CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

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Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1934. Published at 500 So. 5th St., Minneapolis, Minn. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

Vol. 33 November, 1956 No. 6

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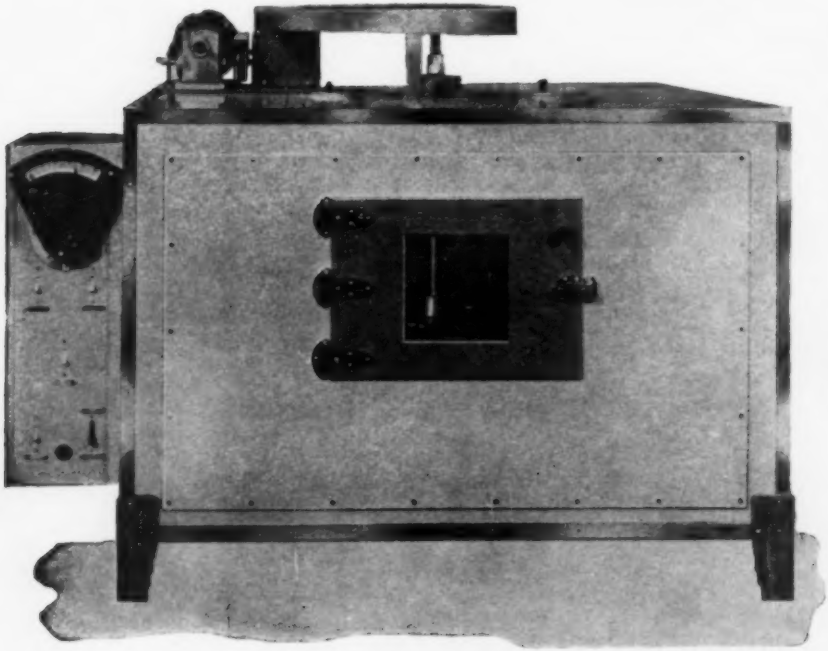

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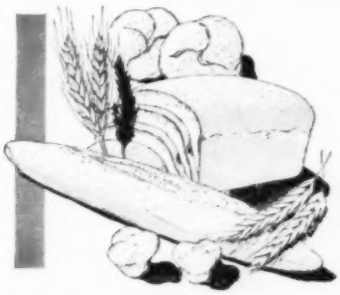


A Brief History of Milling and Baking by SCIENCE WRITER

CHAPTER X. *When New York and Philadelphia Were Milling Capitals*

THE DUTCH IN NEW AMSTERDAM

Nature blessed New York City with a wonderful harbor which is connected by sheltered waterways with parts of New England, New Jersey and upstate New York. The Dutch, who established New Amsterdam early in the 1600s, quickly realized the value of these geographical assets and, with typical foresight, acted.

Settlements grew up in Manhattan along the Hudson River as far north as Albany, on the flat lands in New Jersey and Long Island, and even at Hartford in Connecticut. Wheat growing was an important activity of the farms in the areas controlled by the Dutch. Soon wind and water mills appeared on Manhattan Island, now the heart of New York City, to process the grain which was shipped by the inland water routes to that center. The first was built in 1626.

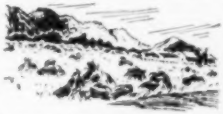
Milling was an extremely important part of New Amsterdam's commercial activity. This is not strange, however, as the center of wheat milling in those days was always close to the center of grain production. And, let's not forget that milling has been a necessary human activity since prehistoric times. Man is just not able to use raw wheatberries for nourishment.

THE BRITISH IN NEW YORK

In 1664 sovereignty over New York passed from the Dutch to the British. At about that time the city became an important milling center and wheat market. In fact milling bulked so large in the city's affairs that it was reflected in the design of its coat-of-arms. Even today the seal of the City of New York displays the sails of a windmill and two flour barrels. Wheat, flour and bread continued to be a vital part of New York's domestic and overseas commerce through the American Revolution and the Napoleonic period.

PHILADELPHIA, THE COLONIES' MAJOR CITY

Prominent as New York was in Colonial times it was not then the leading American city. That distinction belonged to Philadelphia. Leadership as a milling center passed from the former to the latter.



Because of a combination of fertile soil and thrifty, hard-working farmers, the lands around Chesapeake Bay and the Delaware River became the chief center of wheat growing in the colonies and in the world. Philadelphia, Baltimore, Wilmington and Richmond shared the milling and trading activity. After 1750 Philadelphia held the leadership in milling wheat for many years only to be overshadowed by Baltimore in the early 1800s.

WHITE FLOUR . . . WHITE BREAD . . . ENRICHMENT

Throughout this series of articles three themes are apparent: 1.) The milling of grains is an ancient and necessary activity because people cannot eat whole wheatberries. 2) When wheat is milled to the fineness and whiteness demanded by today's consumers certain valuable elements are unavoidably lost. 3.) To overcome inescapable losses the vitamins thiamine, riboflavin and niacin and the mineral iron are restored to the flour or bread through the widely accepted practice of enrichment. Today's white flour and bread meet today's taste —and today's requirements for good nutrition.

The next chapter titled: "Milling Begins Moving Westward" will be published soon.

This is one of a series of articles which is being published in professional nutrition and dietetic journals, and which will be widely distributed for educational purposes. Reprints of this and all previous chapters are available without charge. Write to the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

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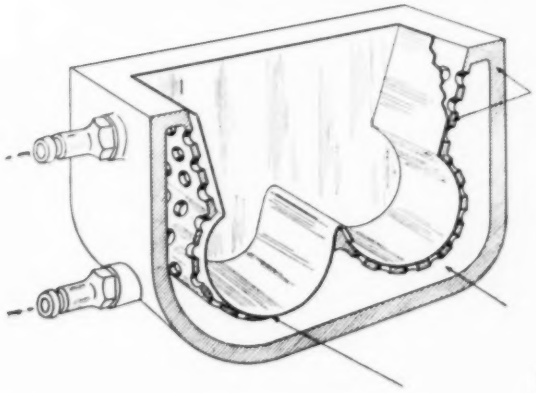

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CEREAL CHEMISTRY

VOL. 33 NOVEMBER, 1956 NO. 6

STRUCTURE OF THE MATURE WHEAT KERNEL
I. Gross Anatomy and Relationships of Parts[1]

DOROTHY BRADBURY, IRENE M. CULL, AND M. M. MACMASTERS[2]

ABSTRACT

A kernel of wheat is a dry, one-seeded fruit. It has on one surface a crease that, in the commonly grown varieties, extends inward nearly to the center. The pericarp (fruit coat) envelops the seed and is fused with the thin seed coat. Together they form two protective layers around the endosperm and the germ. The projecting lower tip of the germ is especially vulnerable to mechanical injury during harvesting and handling, and is often broken to expose germ tissue. When tissues beneath the seed coat are exposed, they are more readily entered by moisture, molds, etc., than when seed coat and fruit coat protect them.

The pericarp and the outermost tissues of the seed, including the aleurone layer, compose the bran. There is no natural line of cleavage between bran and starchy endosperm. This fact accounts for some of the difficulties encountered in separating the two during flour milling. The germ is, structurally, a separate entity; a separation of germ and endosperm should require no breaking of cell walls.

Wheat is used mainly for the production of flour by milling. This entails separation of bran, endosperm, and germ. Similarly, future expanded industrial utilization of wheat will depend in part upon the separation of structural parts and chemical constituents. All types of processing to which the grain is or may be subjected can be efficiently carried out only if there is a clear understanding of the structural details of the raw material. The grain handler also is vitally interested in the structure of the wheat kernel. Movement of water out of or into the grain is basically dependent upon structure.

Numerous studies of the structure of the wheat kernel have been published, but the material is not readily available to cereal chemists and wheat processors. Many of the best accounts are out of print or published in foreign journals which are sometimes difficult of access. In

[1] Manuscript received July 5, 1956. Presented at the 41st annual meeting, New York, May 1956.

[2] Northern Utilization Research Branch, Peoria, Illinois, one of the Branches of the Agricultural Research Service, U.S. Department of Agriculture.

addition, the terminology for the parts of the kernel is extremely confused and many of the articles are written in technical language and from the point of view of comparative developmental anatomy. Some of the general accounts of wheat kernel structure are those given by Fairclough (9), Gassner (10), Hayward (12), Hector (13), Marimpietri and Tirelli (18), Moeller (21), Percival (22), Tschirch and Oesterle (25), Vogl (26), and Winton and Winton (27). There are also articles dealing with restricted aspects of structure, and with the relation of the structure of the wheat kernel to millability and to the movement of water within the kernel. Only the most pertinent of these will be discussed in connection with descriptions of the parts and tissues involved.

The purpose of the present study, which is presented in a series of four papers including Parts II (5), III (6), and IV (7), is to give an account of the gross and detailed structure of the wheat kernel which will be useful to cereal chemists, grain handlers, and wheat processors. A glossary is appended to Part IV (p. 390), because use of some technical botanical terms is unavoidable.

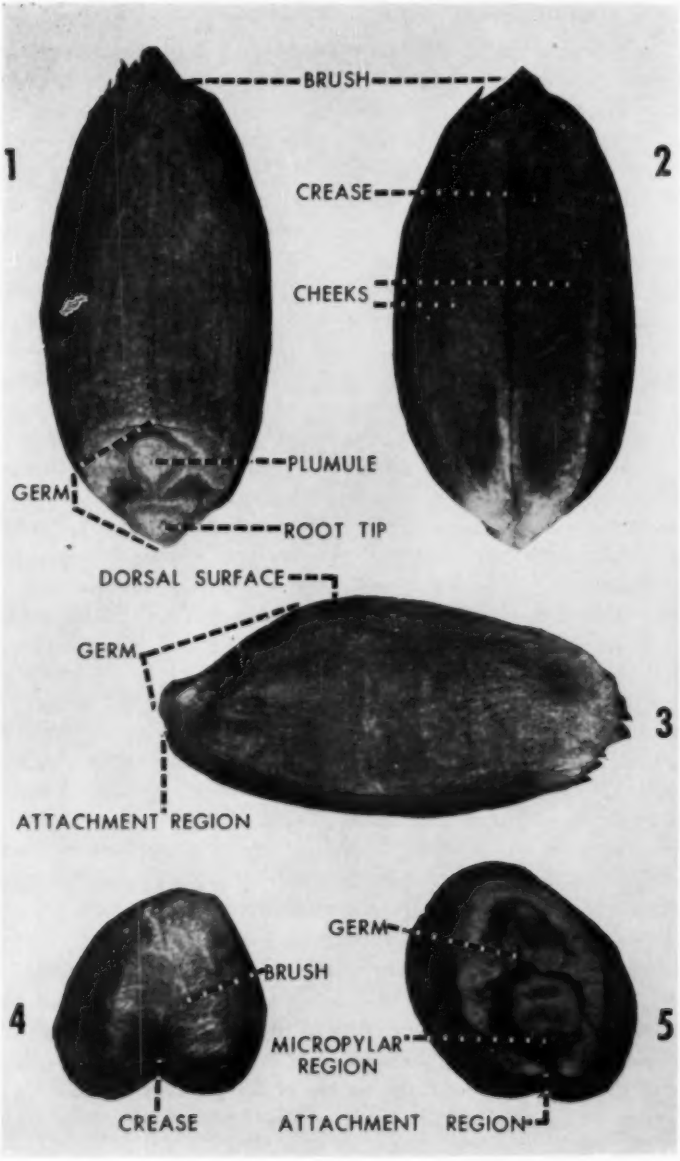
Materials and Methods

The gross anatomy of the wheat kernel was studied from dry, uncut kernels and thick (40–72 μ) freezing-microtome sections of kernels of a hard red winter wheat, Pawnee variety. To obtain sections suitable for photomicrographs, the kernels were steeped 6–14 days in an FAA fixative at 8° C. before sectioning. The fixative contained 50 ml. 95% ethyl alcohol, 5 ml. glacial acetic acid, 10 ml. 37–40% formaldehyde, and 35 ml. distilled water. This treatment reduced the stickiness and stringiness of the gluten. The sections were stained with 0.025% Congo red in aqueous solution buffered to pH 8 with phosphate buffer. They were mounted in glycerol or in the phosphate buffer solution.

Kernels from four classes of wheat were prepared to show the endosperm texture by cutting them above the desired plane of view and then grinding them down on a glass plate which had been abraded with 100-mesh carborundum. This was done to prevent shattering of endosperm cells. Shattering imparts a false appearance of softness, even if the endosperm is actually vitreous in texture. The smooth plane surfaces of the kernels were photographed by reflected light (Fig. 11).

The longisection (Fig. 6) was prepared from photomicrographs by a mosaic method (11) similar to that used in aerial mapping of land.

From 300 to 500 kernels of each of 14 samples of wheat were examined for both shrunken kernels and mechanical injury. Each kernel was handled separately and viewed from all angles through a dissecting microscope equipped with 9× oculars and a 2× objective.



FIGS. 1-5. External views of Pawnee wheat kernels (11×). Fig. 1 — back (dorsal) face; Fig. 2 — crease (ventral) face; Fig. 3 — side; Fig. 4 — brush end; Fig. 5 — germ end.

General Nature and External Appearance of Kernel

The wheat kernel is a dry, one-seeded fruit that does not split open at maturity to shed the seed. The seed consists of germ, or embryo, and endosperm enclosed by a nucellar epidermis and a seed coat. A fruit coat (pericarp) surrounds the seed. The fruit coat and the seed coat have grown together during maturation. This type of fruit (a caryopsis) is characteristic of members of the grass family.

The roughly egg-shaped kernel of wheat is from 4 to 10 mm. long; its length is related to variety and to its location in the spike and in the spikelet during development (3). A well-filled kernel of most common varieties is smoothly curved on its dorsal face (back surface, opposite crease), except at the base where the fruit coat is wrinkled over the underlying germ or embryo (Figs. 1, 3, 5). The embryo occupies from less than one-sixth to over one-fourth of the dorsal surface, depending in part upon the variety (3). The kernel of wheat, like those of rye, barley, and oats, has on its ventral face a furrow or crease between two protruding cheeks (Figs. 2, 4). In the commonly grown varieties of wheat the crease actually penetrates nearly to the center of the kernel (Fig. 7), although externally the crease may vary from shallow to deep (3) because its flanks may touch over a small or greater area. The cheeks of the kernel section for Fig. 7 had spread apart during steeping. The crease was produced when the developing seed became filled with stored food and bulged out on each side. The embryo lies at an angle to the upper dorsal surface of the grain (Fig. 3). The lower end of the embryo, in which the root tip is located, projects slightly beyond the attachment region (the place where the kernel was attached to the stem) (Figs. 3, 5). A small dark spot sometimes shows through the fruit coat at the lower tip of the embryo (Fig. 5). This is the area about the micropyle, a very small opening that was present at flowering time in the cell layers that later developed into the seed coat. At the apex or tip of the kernel there is a brush composed of many hairs (Figs. 1, 2, 4).

The color of the kernel is one of its most constant varietal characteristics; length and endosperm texture are the other two (3). Wheats, with the exception of some Abyssinian and durum varieties, are classed as white or red (3). The dark color of the red wheats arises primarily from materials present in the seed coat, but it is influenced by the texture of the endosperm and the nature of the pericarp (3, 22).

Parts of Kernel

Pericarp. The tissues of the pericarp (fruit coat) form a thin protective covering over the entire kernel, and are reported (4, 14, 17) to

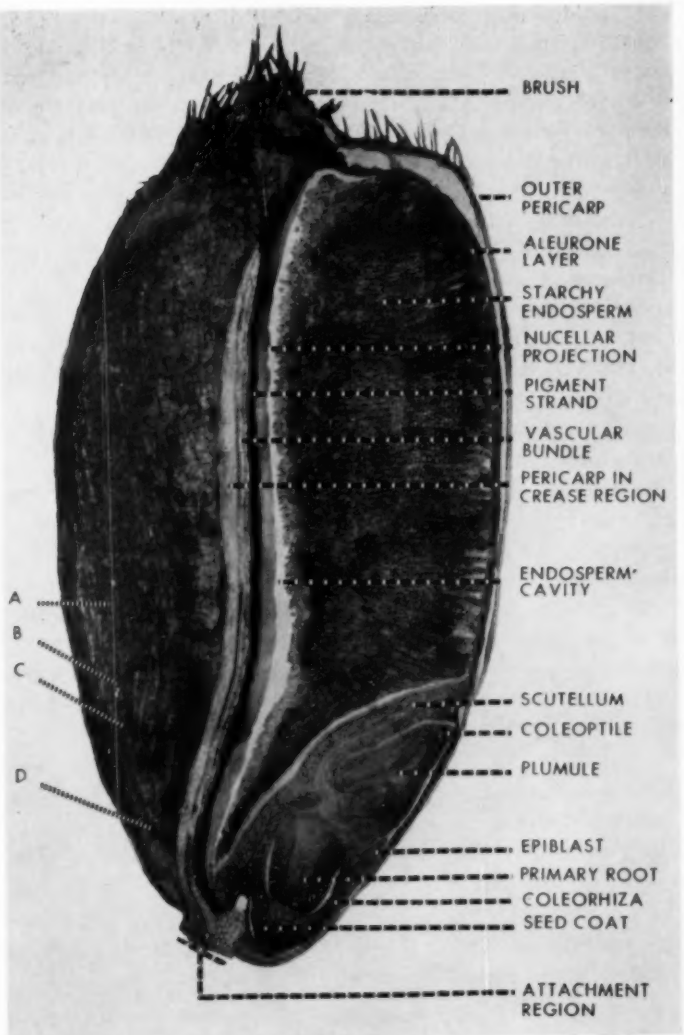


FIG. 6. Pawnee wheat kernel bisected longitudinally through the crease (20×). This is a composite photograph that gives an idealized view of the cut surface at the right and of one flank of the crease at the left.

make up about 4–6% of its weight. The outer surface of the pericarp is covered with a thin cuticle except in the attachment region (Fig. 6). Microscopic examination of the pericarp shows that it is composed of several layers. In order, from outside toward the center of the kernel,

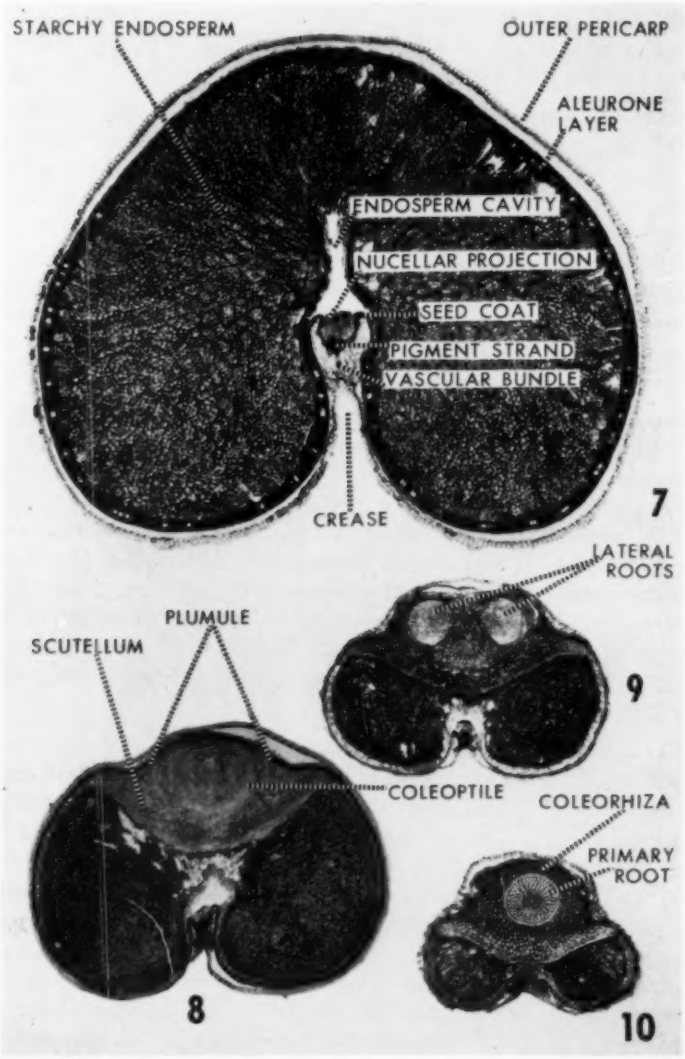
these are: epidermis, hypodermis, remnants of thin-walled cells, intermediate cells, cross cells, and tube cells. The hairs of the brush are extensions of epidermal cells. In water-steeped kernels and in sections of dry kernels mounted in water, the outer part of the pericarp often separates from the inner part (Figs. 6, 7). This separation has its basis in structure, the break occurring in the region of thin-walled cells. The terms outer and inner pericarp will be used to designate the two parts, whether they are separated or united. The outer pericarp is the "beeswing" of the miller. Threadlike hyphae of molds are usually present in the region where the break between inner and outer pericarp occurs. In the crease region, the pericarp contains a vascular bundle (Figs. 6, 7). It extends from the attachment region to near the apical end of the crease. Through its cells water and nutrient material were carried to the developing seed from the wheat stem.

Seed Coat and Pigment Strand. The thin seed coat forms a nearly complete covering over the embryo and endosperm. It is firmly united with the innermost cells of the pericarp. At the bottom of the V-shaped crease the seed coat from each flank of the crease joins a strand of tissue that appears nearly circular in cross section. In red wheats this strand, like the seed coat itself, contains dark-colored pigments and so has been often called the pigment strand (Fig. 7). The pigment strand runs the length of the crease (Fig. 6) and, in red wheat, is conspicuous in unstained sections of the kernel. The seed coat connects with the apex and base of the pigment strand and along its sides. The two structures together form a protective coat about the seed.

Nucellar Epidermis and Nucellar Projection. The nucellar epidermis is composed of a single row of compressed cells. It lies between the seed coat and the endosperm and is firmly united to both. Because this layer appears bright when seen through the microscope, it is often called the hyaline layer.

The nucellar epidermis joins a band of cells that will be referred to as the nucellar projection (Figs. 6, 7). This band lies just interior to the pigment strand and runs parallel to it.

Endosperm. This food-storage tissue is responsible for slightly over 90% of the total weight of the kernel (4). The outermost row of thick-walled cells is the aleurone layer (Figs. 6, 7), which usually makes up about 6–7% of the kernel weight (4, 14). It is the innermost layer of the bran; the latter comprises about 13–16% of the kernel by weight (4, 14, 17). The aleurone layer contains no gluten or starch but has reserve foods in the form of oil and aleurone (protein) granules. The major portion, the starchy endosperm (Figs. 6, 7), is composed of cells



Figs. 7–10. Transections of Pawnee wheat kernels at planes A, B, C, and D, respectively, of Fig. 6. Fig. 7, 30×. Figs. 8–10, 20×.

that contain many starch granules embedded in a matrix of proteinaceous material.

A conspicuous cavity which varies in shape and size is present adjacent to the endosperm in the vicinity of the nucellar projection (Figs.

6, 7). It will be referred to as the endosperm cavity. This cavity contains a substance that swells in water and that can be stained with Congo red and with picric acid.

Differences in texture of the starchy endosperm can be seen in cut kernels with the unaided eye. Some endosperm is hard, translucent, and vitreous or horny in appearance, whereas some is soft, white, mealy, floury, or chalky. These extremes are illustrated in Fig. 11. The texture is related to the denseness of the tissue; the cells of the horny or vitreous endosperm are completely filled with starch and proteinaceous material packed together in a solid mass; the cells of mealy or floury endosperm have many small spaces around the starch granules or separating the cell contents from the wall. Percival (22) also noted the development of fissures between cells.

Endosperm texture is one of the most constant characteristics associated with different classes and varieties of wheat (3) (Fig. 11). The endosperm of durum or macaroni wheats is typically very hard and translucent, this character being little affected by environmental conditions. The soft red winter wheats usually have a soft or mealy endosperm that appears white when photographed. Hard red wheats have a combination of vitreous and soft endosperm or have a hard endosperm unless they are grown under humid conditions or in soil deficient in nitrogen. In kernels that show endosperm of both textures, the apical portion is always horny and the mealy part is usually located near the embryo (22).

Germ. The germ or embryo, usually about 2–3% of the kernel by weight (4, 14), is partly embedded in the endosperm at the base of the kernel. It is rich in oil and protein. The embryo is composed of two major parts, the embryonic axis which at germination develops into the seedling, and the scutellum which nourishes it. In some literature references the term embryo is used inexactly to mean only the embryonic axis. The embryonic axis is composed of the shoot (plumule) pointing toward the brush end of the grain and the primary root pointing toward the base (Fig. 6). Protective sheaths cover these delicate parts; the coleoptile sheathes the plumule; the coleorhiza covers the primary root (Figs. 6, 8, 10). Already differentiated in the plumule are several foliage leaves that surround the growing point of the stem. The parts of the primary root are well defined. Two pairs of secondary rootlets have been formed, but because of their lateral position they are not visible in a median longisection of the kernel cut parallel to the crease. The lower pair of roots shows clearly in a transection through the embryo (Fig. 9). Resumption of growth of the partially

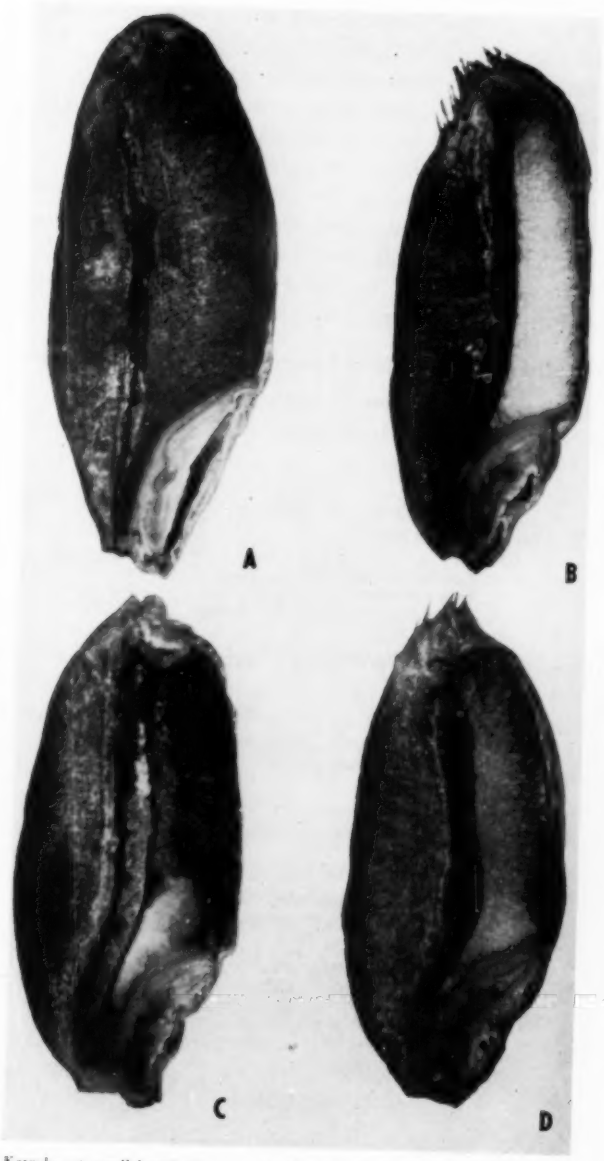


FIG. 11. Kernels cut parallel with the crease to show texture of endosperm in four classes of wheat (11×). A, durum, Stewart 221. B, soft red winter, Trumbull. C, hard red spring, Mida. D, hard red winter, Pawnee.

developed plumule and primary and lateral roots produces the stem, leaves, and part of the root system of the wheat seedling.

Attached to the side of the embryonic axis nearest to the endosperm is the shield-shaped cotyledon or scutellum of the embryo (Figs. 6, 8). Its convex face is embedded in the endosperm; its slightly concave surface partly encloses the embryonic axis.

Opposite the scutellum, on the other side of the embryonic axis, there is a small scalelike outgrowth, the epiblast (Fig. 6). According to Avery (1) it has little morphological significance.

The parts and tissues of the wheat kernel and their relationships to each other are summarized in Table I.

Condition of Kernels in Commercial and Pure Samples

Any careful consideration of the relationship of structure of the kernel to absorption or loss of water or to storage without molding

TABLE I

PARTS OF THE WHEAT KERNEL AND THEIR RELATIONSHIPS TO EACH OTHER

- Wheat kernel (caryopsis)
  - Pericarp (fruit coat) — Bran
    - Outer
      1. Epidermis (epicarp)
      2. Hypodermis
      3. Remnants of thin-walled cells
    - Inner
      4. Intermediate cells
      5. Cross cells
      6. Tube cells
  - Seed
    - Seed coat (testa, spermoderm, tegmen) and pigment strand
    - Nucellar layer (hyaline layer, perisperm) and nucellar projection
    - Endosperm
      1. Aleurone layer
      2. Starchy endosperm
    - Germ (embryo)
      - Scutellum (cotyledon)
        1. Epithelium
        2. Parenchyma
        3. Provascular tissues
      - Embryonic axis
        - Plumule, covered by coleoptile
        - Primary root, covered by coleorhiza
        - Secondary lateral rootlets
      - Epiblast

TABLE II

CONDITION OF KERNELS IN FOURTEEN SAMPLES OF WHEAT[a]

DESCRIPTION OF SAMPLE[b]	FIVE ARBITRARY CATEGORIES USED IN CLASSIFICATION				
	Kernels Shrunken	Pieces of Kernels	Pericarp and Seed Coat Broken[c]	Pericarp Severely Broken	Pericarp with Slight Breaks or None
	%	%	%	%	%
Composite samples from terminal markets, Grade 1, dockage-free					
DHW 1949	0.0	5.3	45.3	27.6	21.6
DHW 1951	1.3	6.3	31.3	20.6	40.3
DHW 1951	4.3	8.3	32.6	28.0	26.6
DHW 1951	2.6	6.3	37.6	26.3	27.0
DHW 1951	0.3	11.6	42.0	25.3	20.6
DHW 1951	1.0	3.6	73.0	16.3	6.0
HW 1949	5.0	5.3	26.6	24.6	38.3
HW 1951	2.3	6.0	25.6	25.3	40.6
YHW 1949	0.0	2.6	28.6	28.0	40.6
Pure samples					
Pawnee					
Grade 2 1949	1.2	3.4	18.2	14.2	63.0
Certified 1950	2.4	5.0	16.2	9.6	66.8
Pure 1952	1.5	0.4	18.0	22.0	57.0
Pure 1952	0.4	9.2	21.6	16.6	52.2

[a] Number of kernels examined: commercial samples, 300; Pawnee wheat samples, 500.
[b] DHW, dark hard winter; HW, hard winter; YHW, yellow hard winter.
[c] Endosperm and/or germ showing.

must recognize the fact that many kernels of commercial lots of wheat have breaks in the protective coats. The importance of embryo exposure has been studied by Mead *et al.* (19). Some indication of the amount of mechanical injury suffered by domestic wheat before it reaches the market is given in Table II.

The data show that 25–73% of the kernels of commercial lots of wheat had endosperm and/or germ exposed in consequence of mechanical injury to the pericarp and seed coat. As would be expected, the percentage of similarly damaged kernels was found to be smaller in pure samples that were obtained chiefly from State Agricultural Experiment Stations. The germ end of the grain is particularly vulnerable because of the protruding germ tip. Mead *et al.* (19) reported a direct relationship between the length of projection of the embryo and the amount of mechanical damage done to the embryo.

Discussion

Consideration of the gross structure of the wheat kernel shows why certain difficulties are encountered in cleaning, tempering, and milling wheat. The presence of the crease in the kernel adds materially to the task of cleaning the grain. As pointed out by Miller (20), in many

varieties the flanks of the crease are usually not separated from each other as seen in a water-mounted section (Fig. 7), but lie close together.

Successful tempering of the grain is related to the structure of the various layers covering the endosperm, because toughness of bran and mellowness of endosperm are dependent upon the entrance and the proper distribution of moisture. The relation of structure to water absorption will be discussed in Part II (page 357), after the structure of the protective coats has been described in detail (5).

The gross structure of the kernel suggests one reason why the separation of bran from the starchy endosperm is difficult. There is no natural line of cleavage between the inner layer of the bran (aleurone layer) and the starchy endosperm. In fact, the aleurone layer, since it is the outermost cell-layer of the endosperm, is an integral part of that central body and is intimately joined to the starchy endosperm cells. More knowledge of the microscopic structure of the kernel in this critical area might lead to improvements in milling methods and consequent higher extraction of white flour.

The percentage of flour extraction has been thought to be influenced by the percentage volume of starchy endosperm, which in turn is affected by size and shape of grain, thickness of bran, and size of germ (2). Shellenberger and Morgenson (23) studied bran thickness and flour yield of four varieties of hard red winter wheat. They reported slight but significant differences in thickness of the bran layer but no correlation between bran thickness and flour yield. Crewe and Jones (8) measured bran thickness of three types of wheat and concluded that all had brans of similar thickness. According to Larkin *et al.* (16) there appears to be no relation between thickness of either the entire bran or of any one of its various layers and the milling quality of the Pacific Northwest wheats.

Embryo exposure resulting from injuries received by the grain during harvesting and subsequent handling increases the chances for damage by mold growth during storage (19, 24). Exposure of the embryo and/or endosperm may also cause lack of uniformity in the response of individual kernels to tempering. Jones and Baker (15) found that wheats with a high percentage of grains with exposed germs produced more foam during washing than did those with a low percentage.

Acknowledgments

The authors are indebted to R. W. Haines for taking the low-power photomicrographs of entire kernels and the mosaic photomicrograph of the longisection of the wheat kernel; to R. A. Larkin for the photographs in Fig. 11; to several State Agricultural Experiment Stations for wheat samples; and to J. E. Hubbard for collecting, sampling, and cleaning the wheats used.

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STRUCTURE OF THE MATURE WHEAT KERNEL
II. Microscopic Structure of Pericarp, Seed Coat, and Other Coverings of the Endosperm and Germ of Hard Red Winter Wheat[1]

Dorothy Bradbury, M. M. MacMasters, and Irene M. Cull[2]

ABSTRACT

From the outside inward, the pericarp of hard red wheat is composed of epidermis, hypodermis, thin-walled cells, intermediate cells, cross cells, and tube cells. The cuticularized epidermis and the hypodermis of 1–2 cell-layers form a compact outer covering, the beeswing. The region of crushed and fragmentary thin-walled cells affords a pathway for quick movement of water. In this region mold hyphae are usually present. Intermediate cells are largely confined to the brush and germ ends of the kernel; tube cells to the back surface. Over most of the kernel, cross cells are closely joined. Intermediate cells, cross cells at the germ and brush ends of the kernel, and tube cells form porous tissues because of many intercellular spaces. These tissues connect with a spongy parenchyma tissue in the attachment region at the base of the kernel and provide an easy route for rapid movement of water.

Interior to the pericarp lies the cuticularized seed coat joined with a corky pigment strand that runs the length of the crease. Together they cover the endosperm and germ. A second covering is provided by the nucellar epidermis joined with a nucellar projection that parallels the pigment strand.

The pericarp, seed coat, pigment strand, nucellar epidermis, and nucellar projection enclose the endosperm and germ of the wheat kernel. Together they form about one-half of the bran. Because of their location and structure they give mechanical protection, affect the absorption and loss of water and solutes, and hinder to some extent the penetration of molds.

The significance of the structure of these parts of the kernel to milling problems lies chiefly in the way it affects absorption of water and its passage into the endosperm during tempering and conditioning. The structure facilitates separation of large flakes of bran from the endosperm and germ. Characteristics influencing water absorption are also

[1] Manuscript received July 5, 1956. Presented at the 41st annual meeting, New York, May 1956.
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important to problems connected with the drying of grain.

A glossary of botanical terms used in this paper is given following Part IV, p. 390.

Materials and Methods

Mature hard red winter wheat, Pawnee variety, 1949 and 1950 crop years, was used. The preparations varied with the tissue studied and the information sought.

Frozen Material. Air-dry kernels were steeped in water and sectioned at 20–40 μ on the freezing microtome. The sections were usually stained with 0.1% Congo red in an aqueous solution buffered to pH 8 with phosphate buffer (Figs. 1, 16, 17, 19, 23, 25, 38). Preparations for Figs. 18 and 22 were stained with Sudan black B and Sudan IV respectively. Sections were mounted in the stain, in the buffer solution, in water, or in glycerol; the mounting medium had little effect on the appearance of the sections.

Paraffin-Embedded Material. Much of the material was prepared for fixation by steeping the kernels in water at 8° C. for 24–48 hours and then slitting the cheeks with a razor blade. Two methods of fixation and dehydration were commonly employed: 1. Material was fixed in a solution composed of 90 ml. 70% ethanol, 5 ml. glacial acetic acid, and 5 ml. formaldehyde (37–40%). The fixed material was dehydrated by a gradual ethanol-chloroform schedule. Paraffin infiltration was accomplished in 5–6 weeks. 2. Material was fixed, dehydrated, and embedded according to the Craf fixation and dioxane-normal butyl alcohol schedule given by Sass (30).

To facilitate sectioning, blocks trimmed to expose the tissue were soaked in a mixture of 20 ml. glacial acetic acid and 80 ml. 60% ethanol (11) for 3–5 days, then kept in an atmosphere of 100% relative humidity for 2 days at room temperature and 2–4 days at 8° C. Sections were cut 10–14 μ thick. Untrimmed blocks that had been in water for 5 years cut much more easily and gave better sections.

Some sections were stained with safranin and fast green (Figs. 24, 26, 27); others with Bismarck brown and fast green (Figs. 35, 36); a few with Sudan black B (Fig. 30). Sections for Figs. 2, 10, 21, 28, 29 were stained by an iron alum-haematoxylin schedule. The sections were mounted in balsam, with the exception of the one for Fig. 30 which was mounted in glycerol. Cell walls are thinner in sections mounted in balsam, which requires a preceding dehydration, than in those mounted in glycerol or in aqueous solutions.

Dissected Material. Many preparations were made from tissues dissected from the kernel or obtained by special treatment. Kernels were

soaked in solutions of sodium or potassium hydroxide to facilitate separation of the layers of the pericarp (Figs. 3, 7–9, 11, 20, 31, 39). Alkaline solutions cause some swelling of the cell walls and tissues.

Many preparations of the seed coat were made from kernels placed in 54% sulfuric acid after removal of one end. A month later the sac-like remains, composed of seed coat and pigment strand, were washed in water and preserved in 70% ethanol until mounted (Figs. 34, 37). Figure 34 was stained with basic fuchsin; Fig. 37 with Sudan black B. For Figs. 32 and 33, 30 μ sections of fresh material were treated with 72% sulfuric acid for 4–6 days. The preparation for Fig. 32 was stained with crystal violet and Congo red; that for Fig. 33 was treated with Clorox, washed, and stained with Sudan black B.

Some parts were dissected from kernels that had been soaked in water at room temperature for 1 to a few hours (Figs. 4, 6, 12–15). Figs. 4 and 14 were stained with Congo red; Figs. 12, 13, and 15 with Bismarck brown; Fig. 6 with iodine-potassium iodide solution.

Cells pictured in Fig. 5 and in Fig. 12 inset were obtained from tissues immersed 5–8 hours in Jeffrey's fluid (10% aqueous chromic acid and 10% aqueous nitric acid solutions, 1:1), washed thoroughly, and stained with Bismarck brown.

Histochemical Tests. The substances tested for and the stains and reagents used are listed. 1. Fats and suberized or cutinized membranes: saturated 70% ethanol solution of Sudan IV or Sudan black B for 20 minutes; 50% ethanol to rinse; glycerol for mounting. 2. Cellulose: iodine-potassium iodide solution followed by 72% sulfuric acid. 3. Lignin: solution made on the slide by pulverizing a small crystal of phloroglucinol in concentrated sulfuric acid. 4. Pectic material: dilute aqueous solution of ruthenium red (approximately 1:10,000; distilled water added to a few crystals to give a clear reddish-pink solution); water for washing after 20 minutes, glycerol for mounting. 5. Tannins: 10% aqueous ferric chloride solution plus a little sodium carbonate.

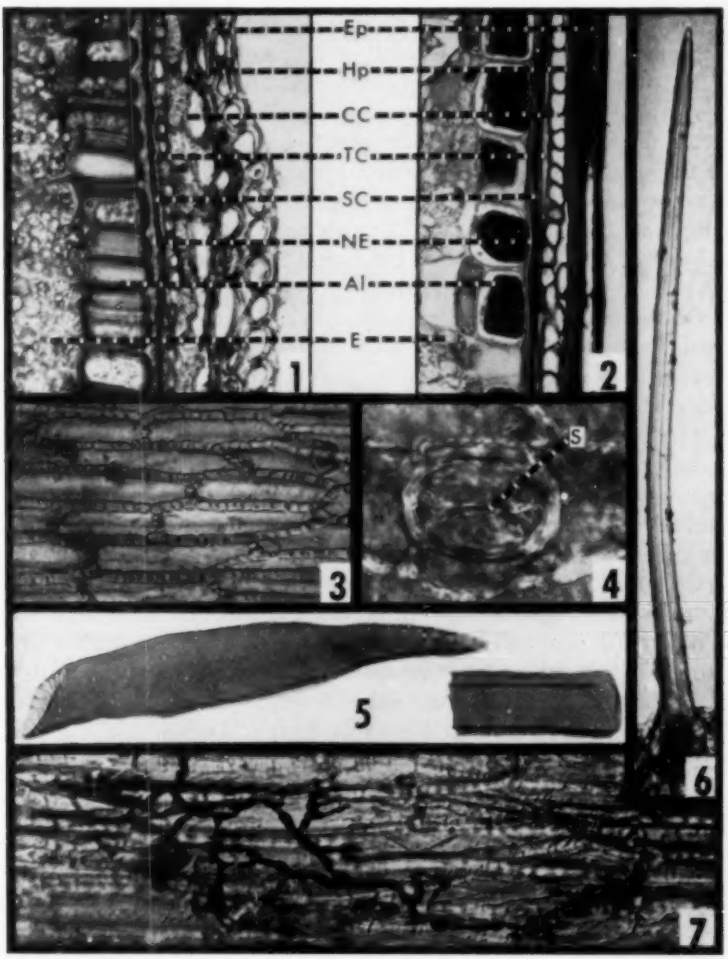
Microscopic Structure

The descriptions of Pawnee wheat have been supplemented by observations of other workers on various wheat varieties. Common varieties of wheat are similar in the structure of most parts. A marked difference exists, however, between the seed coats of red and white wheats (19 and others).

The pericarp completely encloses the seed. From the outside inward the pericarp consists of the following tissues: epidermis, hypodermis, thin-walled cells, intermediate cells, cross cells, and tube cells. Inward from the pericarp the seed coat and pigment strand and the nucellar

epidermis and nucellar projection cover the seed and surround the endosperm and germ. Some of the tissues are shown in Figs. 1 and 2. The cells composing the pericarp tissues contain only remnants of the cell contents.

Epidermis and Hypodermis. The epidermis and hypodermis make up the outer pericarp (beeswing) (see Part I). The epidermis is a single



FIGS. 1 to 7. Figs. 1 and 2 — Transection and longisection through pericarp and adjacent tissues. Ep, epidermis; Hp, hypodermis; CC, cross cell; TC, tube cell; SC, seed coat; NE, nucellar epidermis; Al, aleurone layer; E, starchy endosperm (200×). Fig. 3 — Surface view of epidermal cells (200×). Fig. 4 — Stoma in epidermis; S, stomatal opening (600×). Fig. 5 — Isolated cells of outer pericarp (200×). Fig. 6 — Hair of brush (200×). Fig. 7 — Mold hypha on inner surface of outer pericarp (250×).

layer of cells that forms the outer surface of the kernel except over the basal area, along which it was formerly united with the parent plant (the attachment region). Directly inward from the epidermis is the hypodermis which, in Pawnee wheat, is composed of one or occasionally two layers of cells. Most of the outer pericarp cells are elongate but many at the brush end are nearly as wide as they are long. The cells are closely joined without intercellular spaces and they are arranged end-to-end with their long axes parallel to the length of the kernel (Fig. 3). Dimensions given for epidermal cells by Percival (28) are 125–210 μ by 25–30 μ; those given by Vogl (36) are 80–300 μ by 28–48 μ.

Walls of epidermal and hypodermal cells are thickened. Some reported thicknesses are: 5–7 μ (28) and 3–4 μ (36). In a surface view of the tissues the side and end walls appear beaded because many thin areas, or pits, are present in them (Fig. 3). End walls are transverse or oblique and are thinner than the side walls. Between adjacent cells, walls parallel to the surface of the kernel have smaller, more scattered pits (Fig. 5) than do the other walls.

On the outer walls of the epidermal cells of Pawnee wheat there is a thin, relatively water-impervious cuticle that is especially delicate over the germ or embryo and for a short way from it toward the brush. Breaks may be present in the cuticle as a result of mechanical injury.

The thickness of the outer pericarp was measured in wet-mounted sections of Pawnee wheat kernels. For each of ten kernels a measurement was taken on the dorsal surface, side, cheek, and crease region of a transection through the middle of the kernel; on the dorsal surface of a transection through the embryo; and at three points on the dorsal surface of a longisection. The mean of 80 measurements was 34 μ. The thickness varied greatly on each kernel, but was unrelated to location on the kernel. The variations in thickness appeared to be related to the amount of compression rather than to differences in number of cell layers. Epidermal and hypodermal cells were sometimes so compressed that their cavities were barely evident. Data of Larkin *et al.* (21) show that the mean outer pericarp thickness of three varieties of soft and semihard Pacific Northwest wheats was 28.8 μ, 33.5 μ, and 29.5 μ. The authors stated that the outer pericarp was thickest toward the brush end but otherwise approximately uniform in thickness.

Stomata are sometimes present in the epidermis on the flanks of the crease; they are most plentiful near the brush end of the kernel (Fig. 4).

At the apex of the kernel many of the epidermal cells are modified to form the hairs that make up the brush. According to several authors (e.g., 28, 36) some hairs are 1 mm. long, but most are half that length, and some are as short as 120 μ (24). The hairs are bulbous at the base

and taper to the tip (Fig. 6). They are straight or curved. The thickness of the wall usually exceeds the diameter of the lumen or cell cavity, except in the bulbous base where the diameter of the cavity is often as great as or greater than the wall thickness. Some reported extremes of wall thickness are 3 μ and 9.5 μ; of the lumen diameter, 1.5 μ and 6 μ (35, 36).

Thin-Walled Cells. Inward from the hypodermis lie remnants of thin-walled cells. When the outer pericarp is peeled from a water-steeped kernel of Pawnee wheat, some of these are present on its inner surface. The remnants are more abundant near the brush end than on other parts of the outer pericarp. Some early authors called these parenchyma cells, but Winton and Winton (38) considered them to be collapsed inner cells of the hypodermis. Whatever their origin, these cell remnants facilitate movement of water and favor the separation of the outer from the inner pericarp. Fungal hyphae are commonly found in abundance between the outer and inner pericarp (Fig. 7). Judging from what has been reported by Christensen (7), the majority of them are probably dead.

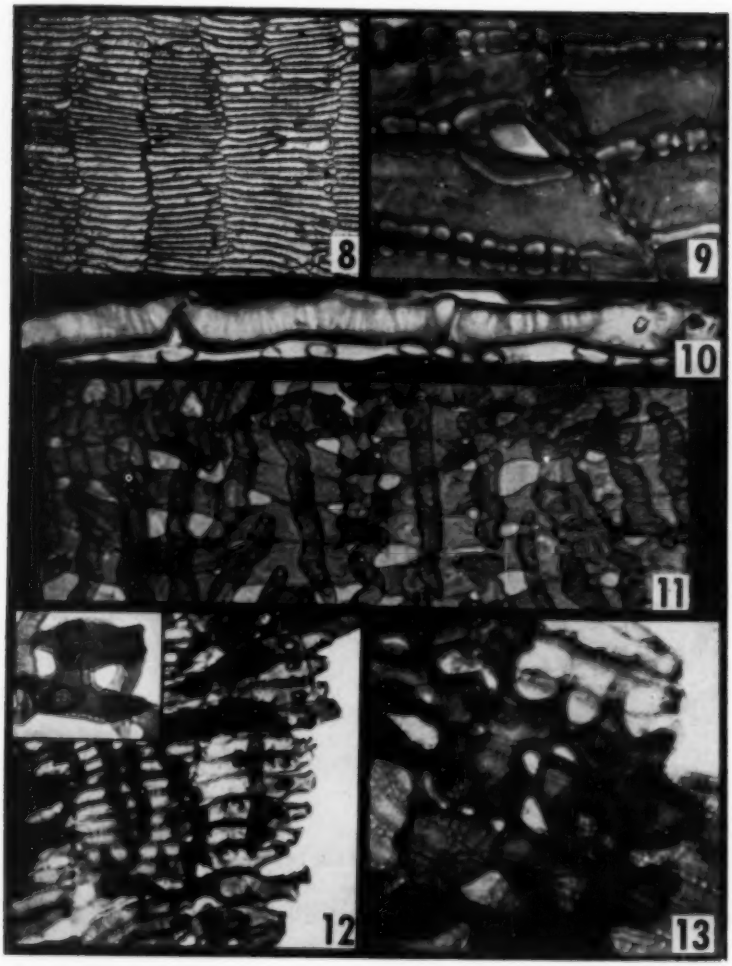
Intermediate Cells. In some areas of the kernel, especially at the brush end and in the vicinity of the germ, intermediate cells (35) lie just beneath the remnants of the thin-walled cells. Winton and Winton (38) described and figured these irregularly shaped cells, but many investigators have made no mention of them.

In both the brush and germ areas the intermediate cells are somewhat flattened and are joined to each other by their projections to form a rather open tissue (Figs. 13–15). The walls between adjoining cells are pitted.

The intermediate cells near the embryo possess many projections by which they are united with other intermediate cells and with cross cells (Fig. 15). More than one layer of intermediate cells was observed over the edge of the germ in Pawnee wheat kernels.

Cross Cells. Over most of the kernel's surface a layer of cross cells lies directly interior to the remnants of thin-walled cells. Cross cells are elongate and are closely joined side by side to form rows that run lengthwise of the kernel (Fig. 8). The long axes of the cells run perpendicular to the long axis of the kernel; hence, "cross cells." Intercellular spaces are small or lacking. Some remnants of the former cell contents are often conspicuous in stained pieces of the tissue.

Cross cells are shaped like tubes that have become slightly flattened by compression; consequently they are often somewhat rectangular in cross section (Fig. 2). According to Percival (28), these cells are about



FIGS. 8–13. Fig. 8 — Surface view of cross cell layer (100×). Fig. 9 — Detail of Fig. 8; note pitted walls and intercellular space (1000×). Fig. 10 — Cross cells showing face view of pitted walls (500×). Fig. 11 — Tissues from over embryo; cross cells running horizontally, tube cells running vertically (200×). Fig. 12 — Tissues from over edge of embryo; cross cells of common type at left, of irregular type at right (150×). Insert shows two enlarged cross cells of irregular type; two tube cells in background (200×). Fig. 13 — Intermediate cells at brush end of kernel; cross cells in background (300×).

100–150 μ by 15–20 μ. Some investigators report extreme lengths of 200–300 μ (e.g., 35, 36). The average thickness of cross cells of Pawnee wheat on the sides, cheeks, and flanks of the crease in about the middle of the kernel was found to be 10.1 μ. A few measurements of cross cells on the back surface in the middle of the kernel and over the embryo

indicated that the cells were about 50% thicker on the back surface. Alexandrov (1) has pointed out the difference in the shape of individual cross cells in different parts of the kernel of club wheat. He noted that the cells were long and not very thick on the sides of the kernel where filling of the endosperm had exerted the greatest pressure; and that the cells were shorter, thicker, and more bent on the back surface where the pressure had been less. Kudelka (20), describing the rye kernel, called attention to similar differences in the appearance of cross cells in well-filled kernels and in those not well filled.

The side and end walls of cross cells have pits similar to those in comparable walls of epidermal and hypodermal cells (Figs. 9, 10). The end walls are thinner than the side walls.

The cross cells near the brush end of the kernel and those that lie over the lower part of the embryo are shorter, more irregular in shape, and more loosely arranged (Fig. 11, inset Fig. 12). Numerous intercellular spaces are present. There is an abrupt transition at the edge of the germ from the common type of cross cell to the irregular type (Fig. 12). The knoblike projections on the walls of these cells are conspicuous and are characteristic of cross cells from the germ area. Such projections are seldom seen on the outer walls of compactly arranged cross cells.

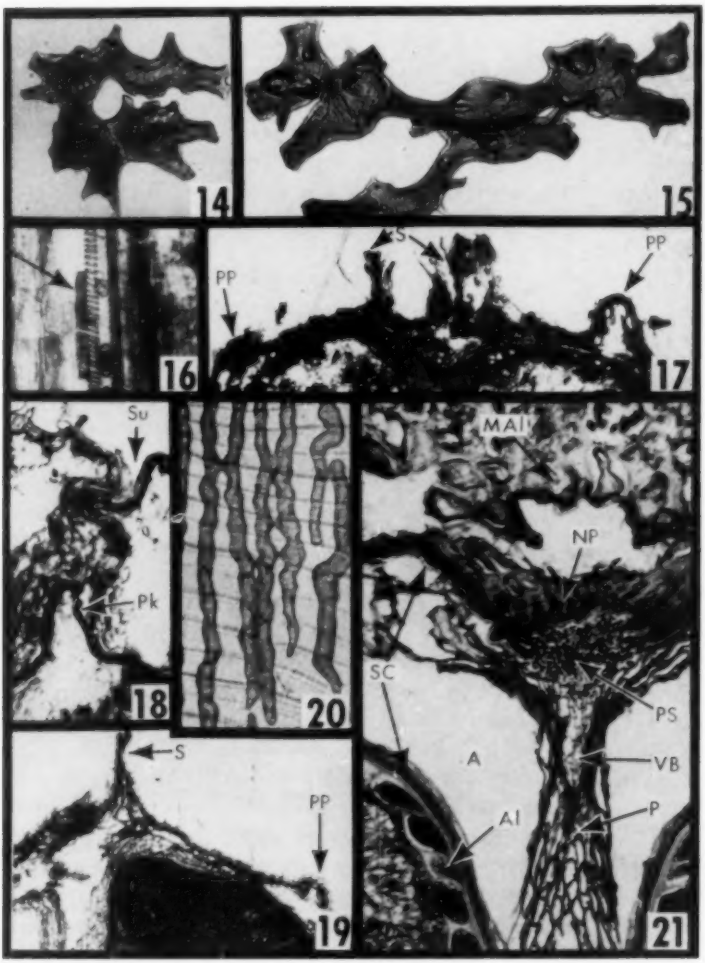
An apparent doubling of the cross cell layer in small areas was observed occasionally near the dorsal surface at the brush end and on the flanks of the crease. Both Moeller (24) and Vogl (36) referred to a double layer of cross cells, but their descriptions suggest that the second layer might have been composed of intermediate cells. Winton and Winton (38) considered the scattered groups of intermediate cells to be remains of an outer cross cell layer.

Tube Cells. Cells of the inner epidermis of the pericarp persist in recognizable form in only a restricted area of the kernel. Because of their long, more or less cylindrical shape they are known as "tube cells." They, like the epidermal and hypodermal cells, run parallel to the long axis of the kernel. In Pawnee variety tube cells occur in a narrow band up the back of the kernel where they gradually spread toward the sides near each end. They are present at the base all over the inner surface of the pericarp around the protruding tip of the germ. Under the brush, also, they are present all over the inner surface of the pericarp.

The tube cells are knobby in outline and are usually joined in only a few places where projections of adjacent cells touch (Fig. 20). The cells are often separated from each other by wide intercellular spaces. The walls are pitted and thinner than walls of cross cells. Percival (28)

gave the dimensions of tube cells as 120–250 μ by 12–15 μ. According to Moeller (24) and Vogl (36), some are 300 μ long. Tube cells at the ends of the kernel are shorter than those in the middle (see Figs. 11, 20).

The foregoing descriptions apply to the tissues of the pericarp over



FIGS. 14–21. Figs 14 and 15 — Intermediate cells from brush end and from over embryo (200×). Fig. 16 — Longisection of vascular bundle in crease region showing xylem elements (at arrow) (200×). Figs. 17, 18, and 19 — Longisections of brush end of kernel: Fig. 17, perpendicular to crease (35×); Fig. 18, parallel to crease (75×); Fig. 19, parallel to crease (35×). S, base of style; PP, projection of pericarp at margin of brush; Su, suture of fruit coat; Pk, peak of seed coat. Fig. 20 — Surface view of tube cells on back of kernel; cross cells in background (200×). Fig. 21 — Transection of kernel in crease region (150×). MAl, modified aleurone layer; NP, nucellar projection; PS, pigment strand; SC, seed coat; VB, vascular bundle; P, pericarp; A, air space; Al, aleurone layer.

the greater portion of the kernel. Variations in the form and structure of the pericarp in a few special regions should be noted.

Pericarp at Brush End. Near the brush on the back of the kernel there is usually an air space between the outer and inner pericarp (Part I, Fig. 6 (2)). This space is perhaps caused by shrinkage of the kernel as it dries during ripening. Remnants of the bases of the two styles are present among the hairs of the brush (Fig. 17). There is a slight elevation of the pericarp at the margin of the brush (Fig. 17). At a right angle to the section shown in Fig. 17, a longisection cut parallel to the crease (Fig. 19) passes through the base of one style and through the margin of the brush on the back surface. The peak of the seed coat and the suture which was formed where the fruit coat closed over the developing seed are seen in a longisection cut between the two styles and parallel to the crease (Fig. 18).

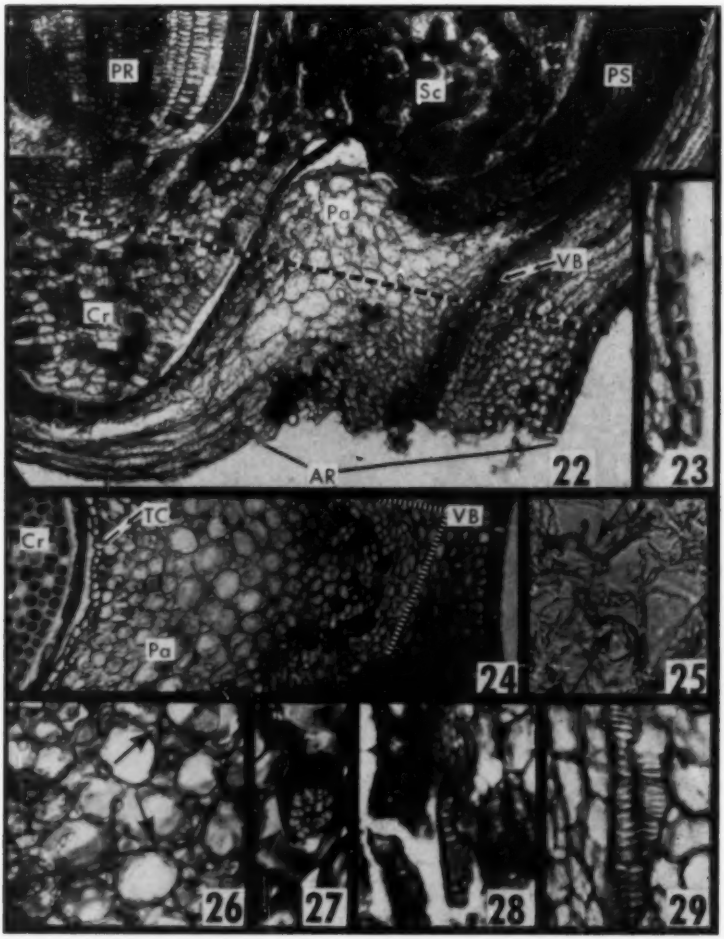
Pericarp in the Crease. The thickness of the pericarp at the bottom of the V-shaped crease and the characteristic air spaces formed in this tissue during maturing of the grain are shown in Fig. 21. A vascular bundle runs through the pericarp tissue from near the tip of the pigment strand to the very base of the kernel (Fig. 21, VB). Xylem elements with ring and spiral wall-thickenings are conspicuous in this bundle throughout most of its length (Fig. 16).

Pericarp at Base of Kernel. Near the base of the kernel, below the end of the pigment strand, the pericarp is thicker than elsewhere. The parenchyma tissue in this region (Figs. 22, 24) is composed of many-sided cells that are joined to each other by short tubular projections (Fig. 26). Many intercellular spaces are present. Mold hyphae are often present in this area (Fig. 25). A surface view of a wall between parenchyma cells shows thin areas which appear unstained (Fig. 27).

The vascular bundle in this basal region has pitted (Fig. 28) as well as spiral xylem elements (Fig. 29). The pitted elements are especially abundant near the ragged surface that marks the attachment region, the place where the kernel was attached to the stem.

The epidermis of this basal region is distinctive in appearance because the cells are about equal in length and diameter (Fig. 23). The attachment region is not covered by an epidermis (Fig. 22), and the walls of its cells do not stain with Sudan IV.

Seed Coat and Pigment Strand. The seed coat and pigment strand together form a complete coat about the seed (Part I). The pigment strand runs the length of the crease. Early in the developmental history of the seed, when the ovule is small, this strand of tissue is much shorter than it is in the mature kernel; its cells transport water and nutri-



FIGS. 22–29. Fig. 22 — Median longisection parallel to crease, at base of kernel (100×): PR, primary root; Cr, coleorhiza; Sc, scutellum; PS, pigment strand; Pa, parenchyma; VB, vascular bundle; AR, attachment region. Fig. 23 — Short epidermal cells at base of kernel (200×). Fig. 24 — Transection at level indicated by dotted line in Fig. 22 (93×): TC, tube cells; rest of code as in Fig. 22. Fig. 25 — Mold hyphae near base of kernel (400×). Fig. 26 — Parenchyma tissue of Fig. 24 enlarged (200×); cells are joined at projections (arrows). Fig. 27 — Pitted area of wall of a parenchyma cell (400×). Figs. 28 and 29 — Pitted and spiral xylem elements from vascular bundle near base of kernel (500×).

ents to the growing seed from the vascular bundle in the pericarp. As the seed matures the cells of both seed coat and pigment strand become filled with an oily material (29) or a corky substance (19). The resistance of the seed coat and pigment strand to sulfuric acid has been considered by several authors to be an indication of their corky nature.

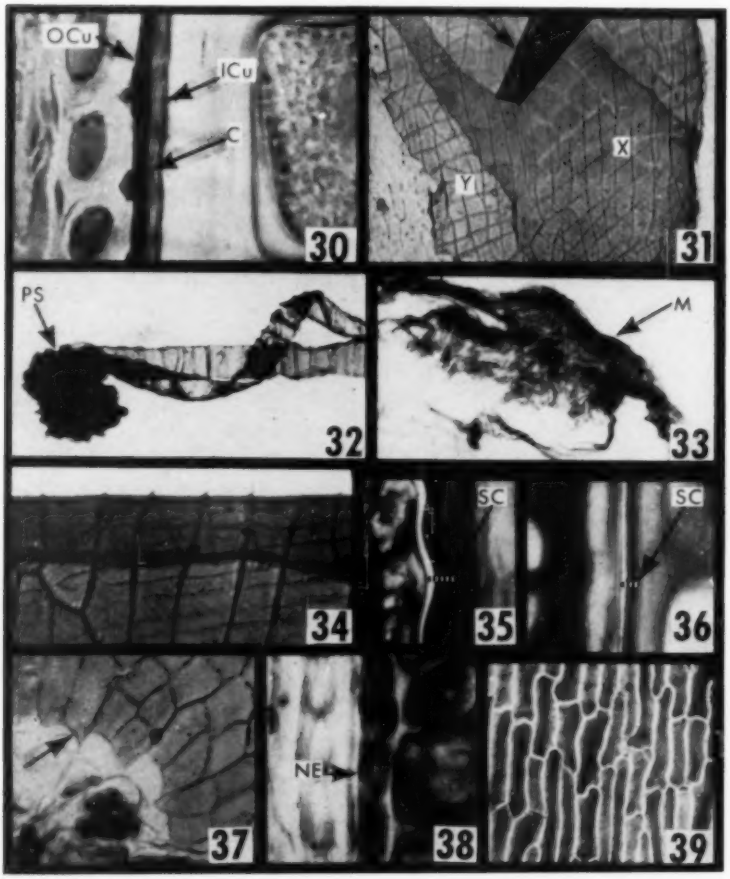
According to Krauss (19), a cutinized wall layer surrounds the corky material in the cells of the pigment strand of Trubilo wheat; the cutinized layer is in turn surrounded by an outer lignified layer.

The pigment strand of Pawnee wheat is composed of cells that are slightly elongated parallel to the long axis of the kernel. In cross section they are irregular in outline and fitted together somewhat like the parts of a jigsaw puzzle (Fig. 21). They contain a golden-brown pigment, and the intercellular wall substance gives a strong positive test for lignin. The pigment strand is not destroyed by treatment with 54% sulfuric acid for a month or with concentrated sulfuric acid for 4 days.

The seed coat of Pawnee wheat is similar in structure and composition to that of the red Trubilo variety described so fully by Krauss (19). The seed coat is located between the pericarp and the nucellar epidermis (Figs. 1, 2) and is firmly joined to either tube cells or cross cells on the outside and to the nucellar epidermis on the inside. With proper staining and magnification, three layers can usually be discerned in a section through the seed coat: a thick outer cuticle, a "color layer" that contains pigment, and a very thin inner cuticle. The inner cuticle may be so fused with the color layer in some places that it is undetectable. In the preparation for Fig. 30, the two cuticles were heavily stained by Sudan black B; this indicates a content of suberin or cutin. Brown (6) described the seed coat of wheat as a mucilaginous layer covered by a thin inner and a thicker outer cuticlelike layer. Shetlar (32) concluded from microchemical tests that the seed coat was cutinized rather than suberized.

A fourth extremely thin layer lies between the outer cuticle and the color layer. It is best observed when sections through the seed coat are treated with ruthenium red, or with iodine-potassium iodide solution followed by 72% sulfuric acid. The layer gives a positive test for both pectic material and cellulose. The color layer of Pawnee wheat, unlike that of the Trubilo variety (19), gives a slight test for tannins.

The structure of the color layer can be observed when the seed coat is viewed from the surface or when it is treated with sulfuric acid. Many investigators have established that the seed coat is derived solely from the inner of the two integuments that cover the ovule (forerunner of the seed). This inner integument is composed of two layers of cells which, in an altered and compressed condition, form the color layer of the seed coat. Most cells of each layer are elongated and have blunt-pointed, oblique, or truncate ends. The cells of the two layers cross each other at an angle of less than 45°. A surface view of the seed coat



FIGS. 30–39. Fig. 30 — Seed coat and adjacent tissues in longisection through cheek of kernel (1000×): OCu, outer cuticle; C, color layer; ICu, inner cuticle. Fig. 31 — Surface view of seed coat and adjacent layers (150×): Outer cuticle rolled back at arrow; cells of color layer seen through cuticle at X; cuticle removed at Y. Figs. 32 and 33 — Seed coat separated into two membranes by acid: Fig. 32, PS, pigment strand (150×); Fig. 33, M, micropylar area (surface view) (250×). Fig. 34 — Outer membrane of acid-treated seed coat; edge rolled back at top to show projections outlining pattern of cells of color layer (500×). Figs. 35 and 36 — Sections through seed coat, SC (600×): Fig. 35, near pigment strand; Fig. 36, side of kernel. Outer cuticle light. Fig. 37 — Outer membrane of micropylar area of acid-treated seed coat; margin of the thick part of the outer cuticle at arrow; membrane stained with Sudan black B (250×). Fig. 38 — Nucellar epidermis, NE, in longisection through crease region (400×). Fig. 39 — Surface view of nucellar epidermis (100×).

(Fig. 31) shows the outer transparent cuticle rolled back (at arrow) and the crossing layers of cells, covered by the cuticle at X and with cuticle removed at Y. Percival (28) stated that the cells are 100–150 μ by 15–20 μ. Vogl (36) reported a width of only 9–12 μ. The average dimensions of cells of the seed coat of Pawnee wheat (determined from 75

measurements in one kernel) were 116 μ by 20 μ for the outer layer and 191 μ by 18 μ for the inner layer. The longest cells were about twice as long as the shortest.

When the seed coat is left in sulfuric acid for some time it separates into two membranes (Figs. 32, 33). This separation is brought about by the hydrolysis of the outer walls of the outer cell layer (19). These walls comprise the thin pectic and cellulosic layer previously mentioned. The outer membrane is merely the outer cuticle, although it appears cellular because its under surface projected into the slight indentations located at the boundaries of the underlying cells. The projections can be seen on the rolled-back edge of the outer membrane (Fig. 34). Krauss (19) reported grooves rather than projections. On this membrane, a crossing layer of cells is suggested by adhering remnants of walls of the cross cells of the pericarp (Fig. 34). According to Krauss (19), these remnants are lignified. In Pawnee wheat, no positive test for lignin was obtained on the outer surface of the cuticle of the seed coat, although walls of cross cells gave a positive test.

Both the outer cuticle and the seed coat as a whole vary in thickness in different parts of the kernel. Judging from averages of measurements in eight kernels of Pawnee wheat, in six areas of transections and in four areas of longisections, the thickness of the outer cuticle is about 2–4 μ and of the seed coat about 5–8 μ. Larkin *et al.* (21) reported the thickness of the seed coat (spermoderm) to be 1.5–3.5 μ, but actually these are figures for the thickness of the outer cuticle. Both outer cuticle and entire seed coat are thickest near the pigment strand in the crease region, at the apex of the kernel below the styles, and at the base of the kernel near the juncture of coleorhiza and scutellum. Thick and thin areas of the seed coat are shown in two successive transections of the same kernel: one in the crease near the pigment strand (Fig. 35), the other along the side of the kernel (Fig. 36). Pugh *et al.* (29) stated that the outer cuticle is thinnest over the embryo. Larkin *et al.* (21) reported variation in the thickness of the outer cuticle (called spermoderm by them) from 1.5 μ to 3.5 μ.

The foregoing description applies to all parts of the seed coat except the micropylar area. At the time of fertilization of the wheat flower the two-layered inner integument covers the tip of the ovule (forerunner of the seed) except for a small canallike opening. The opening leads through the integument from the cavity in which the ovule develops to the central body (nucellus) of the ovule. Through this opening, known as the micropyle, the pollen tube enters.

Two publications (19, 29) give detailed descriptions of the seed coat in the micropylar area. The exact structure of tissues in this region is

difficult to determine because they shrink during drying of the grain. The authors of the two articles were aided in their interpretation of the structure by a study of the immature kernel in which tissues are less compressed and shrunken. According to Krauss (19), by the time the kernel of a red wheat, Trubilo, is 5 mm. long the cuticles of the inner integument (which later becomes the seed coat) have dissolved in the area about the micropyle. This area without cuticles has a diameter of over 82 μ. In both layers of the inner integument the cell cavities are filled with fatty or corky material. The swollen mass of the embryonic appendage (which forms the tip of the root sheath) is the part of the embryo that lies nearest to the micropylar region of the seed coat. Reference will be made in Part IV to the possible significance of this condition (3). Krauss (19) stated that in the ripe kernel the micropylar region of the seed coat joins, on the inside, the nucellar epidermis, whose walls are partly lignified and partly impregnated with corky material.

The following description of the seed coat in the micropylar region summarizes that of Pugh *et al.* (29). At the micropyle the color layer of the seed coat turns outward and folds back upon itself. The margin of this folded-back portion joins the thick outer cuticle of the seed coat. There is, consequently, a somewhat circular area covered by a double color layer. The diameter of this area is approximately 100 μ (from Pugh's figures). The thin inner cuticle of the seed coat is probably present over the surface of the folded-back portion of the color layer.

The descriptions of Krauss and Pugh *et al.*, though of different wheats, are in agreement as to the absence of a heavy outer cuticle over the seed coat in the micropylar region. Pugh *et al.* said that a thin outer cuticle is probably present there; Krauss reported that the seed coat in that region is completely uncutinized.

In unstained longisections of mature kernels of Pawnee wheat the micropylar area can be recognized as a caplike region composed of cells that are less compressed and more deeply pigmented than those in other areas of the seed coat. The thick portion of the outer cuticle terminates abruptly (Fig. 37). A very thin cuticle covers at least part of the remaining micropylar area. The heavily pigmented cells of the color layer made observations in this region difficult. No tissue interior to the seed coat gave a positive test for lignin.

Nucellar Epidermis and Nucellar Projection. The nucellar epidermis (hyaline layer) lies between the seed coat and the aleurone layer and is closely united to both. In the immature seed it is the outermost layer of the nucellus, the central body of the ovule. During maturation

of the ovule into the seed the embryo and endosperm develop within the nucellus and use its tissue for their own nutriment. Eventually only the epidermis of the nucellus and a band of cells (Fig. 21, NP) that runs parallel to the pigment strand remain. Pugh *et al.* (29) considered this band to be "compressed remains of nucellar tissue which did not disintegrate during the process of maturing." Krauss (19) called this band of tissue the "nucellar projection."

The nucellar epidermis is so compressed during the filling of the endosperm with starch that its cellular nature is rarely apparent in trans- or longisections of the kernel. The walls are closely pressed together, practically obliterating the cell cavities. In the crease region, however, the cells are less compressed, and their outlines are sometimes fairly clear (Fig. 38). A surface view of the layer indicates its cellular structure more clearly (Fig. 39).

Krauss (19) reported that the nucellar epidermis is present over the entire kernel of Trubilo wheat. She stated that over the embryonic axis it is a thin, homogeneous, pectin-containing layer; over the scutellum and endosperm it is a thicker, hyaline, cellulosic layer. According to Fairclough (9) the nucellar layer "seems to surround the whole of the grain except for the greater part of the germ." He observed that, in a longisection of the kernel, the layer becomes thinner at the upper tip of the germ and then extends down over six to twelve of the small aleurone cells. In the present study of Pawnee wheat the nucellar layer was not detected over the embryo.

Significance of Structure

Absorption of Water and Solutes. The structure of the pericarp and of the seed coat throws much light on the probable path taken by water as it enters an immersed kernel. The only part of the kernel not covered by a cuticle is the attachment area at the base. In uninjured kernels only this area permits immediate and rapid entrance of water. Not only is the attachment region without a cuticle, but its parenchyma tissue contains many intercellular spaces. Rapid movement of water undoubtedly occurs from this spongy tissue upward through the pericarp in the area of thin-walled cells. Another pathway for quick movement of water is formed by the labyrinth of intercellular spaces among intermediate, cross, and tube cells over the lower part of the embryo and among cross and tube cells along the dorsal surface of the kernel. On the crease side the spongy parenchyma connects with the pericarp tissue in the V of the crease and consequently with the relatively large air spaces which were formed there during maturation of the kernel. Walls of the pericarp cells imbibe water readily, thus increasing absorption.

Kernels of air-dry Manitoba wheat absorb water amounting to about 4% of their original weight in the first minute of immersion (17). Further absorption occurs at a much slower rate which is affected by temperature. While the structure of the pericarp accounts for the rapid initial pick-up of water by an immersed kernel, it is the nature of the seed coat that helps to explain the subsequent slow absorption of water. In 1911 Schroeder (31) concluded that the semipermeable layer of the pericarp of wheat is probably the cutinized seed coat rather than the cellulosic nucellar epidermis. Krauss (19) concluded that absence of cuticles in the micropylar region of the seed coat is one factor favoring the rapid entrance of water at the germ end of immersed kernels. Recent experiments of Hinton (15) indicate that the testa (seed coat) offers more resistance to water entry than does the hyaline layer (nucellar epidermis), aleurone layer, or endosperm. He stated that "the practical significance of the resistance of the testa is that it limits the water taken up by wheat during washing to that which can be absorbed by the pericarp."

The semipermeable nature of the endosperm coverings in small grains has been studied in considerable detail in wheat, barley, and rye (4, 5, 6, 8, 13, 14, 18, 25, 26, 31, 39). A good review of the work published before 1931 is given by Lehmann and Aichele (22). Most investigators have concluded that semipermeability is localized in the seed coat. This semipermeability is important to the milling industry if any chemical substances are added to tempering water. Recent experiments (37) show that when potassium bromate is added to tempering water at a level of 2.5 mg/100 g. wheat, most of it fails to pass through the bran into the endosperm.

During forced drying, water probably leaves kernels by the same route by which it customarily enters.

Protection Against Fungi. Occurrence of mold hyphae between the beeswing (outer pericarp) and the inner pericarp of wheat kernels is well known (16, 27, 33). The observations of Pugh *et al.* (29) indicate that in mature kernels the intact seed coat probably plays some part in preventing invasion by fungi. In their summary they said: "The testa [seed coat] becomes increasingly resistant to penetration by *Gibberella saubinetii* as the grain matures. The degree of resistance of the membranes seemingly is proportional to their thickness. The outer membrane of the testa is the most resistant layer of the kernel." Mechanical injury of the grain during harvesting and subsequent handling undoubtedly increases the chances for penetration of molds (12, 23, 34).

Identification in Milled Products. The microscopic appearance of the cells and tissues here described is useful in identifying bran in various mill-streams. It is also of use in the identification of wheat in feed mixtures. Wheat closely resembles rye in structure, but the outer pericarp cells, brush hairs, and cross cells of these two grains differ in minor details (10, 38).

Acknowledgments

The authors are grateful to Kansas and Oklahoma Agricultural Experiment Stations for the wheat samples used, to J. E. Hubbard for sampling, and to R. A. Larkin for preparation of some of the material and for taking several of the photomicrographs.

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STRUCTURE OF THE MATURE WHEAT KERNEL
III. Microscopic Structure of the Endosperm of Hard Red Winter Wheat[1]

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ABSTRACT

Endosperm of the wheat kernel is composed of an outer layer of aleurone cells and an inner body of starch-containing cells. Flour is obtained industrially from the starchy endosperm.

The typical aleurone layer bounds the outer surface of the starchy endosperm except where the latter is in contact with the scutellum of the embryo and around the endosperm cavity. The living substance of each cell consists of a nucleus and surrounding cytoplasm; the latter contains oil and many aleurone grains. The walls are moderately thick. Protoplasmic connections between adjacent aleurone cells and between aleurone and starchy endosperm cells have been reported by some workers. A modified aleurone layer of thin-walled cells unaccompanied by starchy endosperm extends over part of the embryo. The endosperm cavity present near the crease is bordered by a modified aleurone layer composed of cells with pitted walls.

Inward from the aleurone layer the endosperm cells, with the exception of those collapsed and compressed cells that adjoin the scutellum, are filled with many starch granules embedded in a proteinaceous matrix. The cells are of three types: 1) peripheral, next to the aleurone layer; 2) central, in the centers of the cheeks; and 3) prismatic, located between the other two. Prismatic and central cells contain large lenticular starch granules and small spherical or many-sided granules; peripheral cells contain granules of an intermediate size.

The significance of the structure of the endosperm to milling problems is discussed.

The endosperm makes up 91–92% of the weight of the wheat kernel; its outermost (aleurone) layer accounts for 6–7% and the starch-containing cells for approximately 85% of the whole kernel (4, Part I). To the miller the term endosperm is practically synonymous with starchy endosperm because he considers the aleurone layer to be the innermost layer of the bran. The starchy endosperm, which is the major portion of the kernel and the source of flour, is of paramount value to the milling industry. The aleurone layer forms a covering over the starchy endosperm. It is therefore of interest to the miller to know what role the aleurone layer may have during tempering and conditioning. Because a clean separation of bran from starchy endosperm is one of the major aims in milling, the nature of the union between the aleurone layer and the starchy endosperm is especially worthy of study.

Botanical terms used are defined in the Glossary on page 390.

[1] Manuscript received July 5, 1956. Presented at the 41st Annual Meeting, New York, May 1956.

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Materials and Methods

Kernels from three samples of Pawnee variety wheat were used. Two samples were harvested at maturity; one was harvested when the grain was in the late soft dough stage.

Frozen Material. Water-steeped mature kernels were sectioned, usually 30 μ thick, on the freezing microtome. Most sections were stained with 0.025% Congo red in an aqueous solution buffered to pH 8 with phosphate buffer (Figs. 1–5, 7, 18–23). The preparation shown in Fig. 8 was stained with Sudan IV. Glycerol or the phosphate buffer solution was used as the mounting medium.

Kernels in the late soft dough stage of development were sectioned at 30 μ for Figs. 12–15. The contents came out of most of the cells during the cutting and handling of the sections, and any that remained were removed with needles. The sections were stained with Congo red and mounted in a pH 8 buffered phosphate solution.

Preparations showing the proteinaceous network (Figs. 16, 17) were made from sections cut at 14–20 μ from mature kernels steeped in an FAA fixative (see Part I (4)). The sections were treated with concentrated nitric acid for 3 minutes, washed thoroughly, neutralized with ammonium hydroxide, washed in water, partially dehydrated, stained with a 1% solution of fast green in 95% ethanol or in clove oil, dehydrated and cleared, and mounted in balsam.

Paraffin-Embedded Material. Sections for Figs. 6 and 9–11 were cut 10–14 μ thick from material fixed and dehydrated according to the first method previously given (see Part II (5)). The sections were stained by an iron alum-haematoxylin schedule.

Microscopic Structure

Aleurone Layer. The typical aleurone layer has been described and illustrated by many investigators. It bounds the outer surface of the starchy endosperm except where the latter is in contact with the scutellum of the embryo and around the endosperm cavity (see Part I (4)). On its exterior the aleurone layer is closely joined to the nucellar epidermis. These relationships and the shape of the individual aleurone cells are shown in Fig. 1. In both trans- and longisections through the kernel the aleurone cells appear square or slightly oblong. Elongation is most commonly perpendicular to the surface of the kernel. The thickness of the aleurone layer (measured on a total of 60 aleurone cells in wet mounts of sections of 20 kernels) ranged from 37 to 65μ; the average was 46.9 μ. This figure is in general agreement with an average of 46 μ (range 32–55 μ) found for three English and Canadian wheats

(9), and a range of 46.0–56.8 μ for three Pacific Northwest wheat varieties (22).

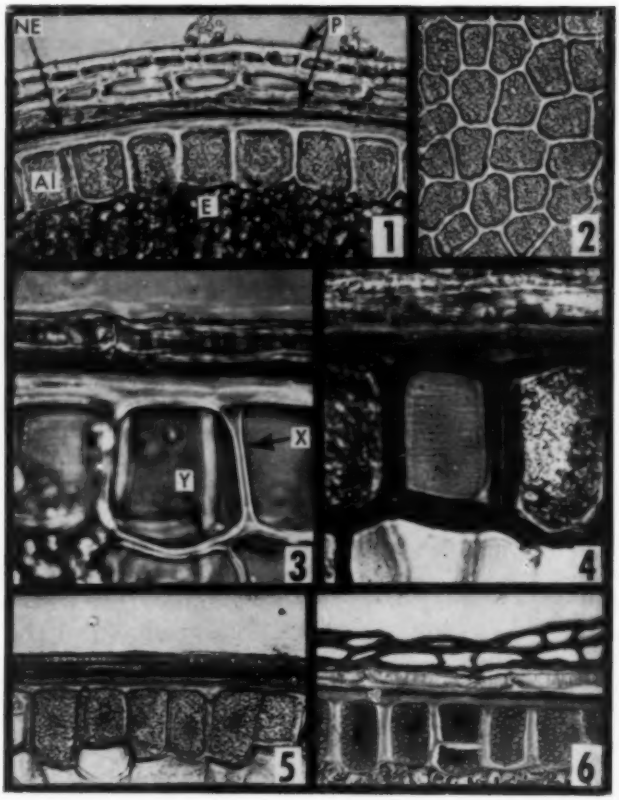
In surface view aleurone cells are 4–7 sided and joined together without intercellular spaces (Fig. 2). They vary greatly in diameter; a range of 25–75 μ was reported by Percival (27).

The walls of the aleurone cells are moderately thick. The approximate thickness of the double wall between adjacent cells in water-mounted sections was given as 8 μ by Tschirch and Oesterle (30); as 6 μ by both Percival (27) and Larkin *et al.* (21). Cobb (8) made an exhaustive study of the aleurone layer of about 50 varieties of Australian wheat and determined for each variety the percentage of area occupied by the cell contents. This percentage varied considerably because of differences in cell size and wall thickness.

The contents of each aleurone cell are surrounded by a wall layer that appears bright or hyaline in sections that are unstained or stained lightly with Congo red (Fig. 3). The intercellular wall material that joins adjacent cells appears darker than the hyaline layer. The cellulosic composition of the hyaline portion is indicated by its birefringence when the wall is viewed between crossed nicols, and by a blue coloration when 72% sulfuric acid is added after sections have been treated with an iodine-potassium iodide solution. The walls between adjacent cells are faintly striated (Fig. 4). Several workers (15, 25, 30, 31) have reported either minute canals or delicate protoplasmic connections between adjacent aleurone cells and between aleurone cells and starchy endosperm cells. Special techniques are necessary to make such connections evident.

Each aleurone cell has a nucleus surrounded by oil-containing cytoplasm in which many small aleurone (protein) grains are embedded. Sections stained to show the nuclei (Figs. 5, 6) also illustrate two other characteristics of the aleurone layer. The innermost boundary of the layer is sometimes conspicuously irregular as a result of variation in thickness of the cells (Fig. 5) (see 9 and 22). Two superposed cells are occasionally present (Fig. 6), although the layer is usually only one cell thick. The cells do not contain gluten and usually contain no starch. Vogl (31) reported the presence of small starch granules in the inner cell of superposed cells. Respiration studies (24) indicate that the aleurone cells are living. According to recent investigations they are rich in B vitamins (18), esterase (11), phytase (26), and proteolytic enzymes (12), but contain no beta-amylase (10).

A modified aleurone layer borders the endosperm cavity near the crease (Fig. 8, MAl). A brief description of this layer has been given by Winton and Winton (32). Both in transections (Fig. 8) and longisec-

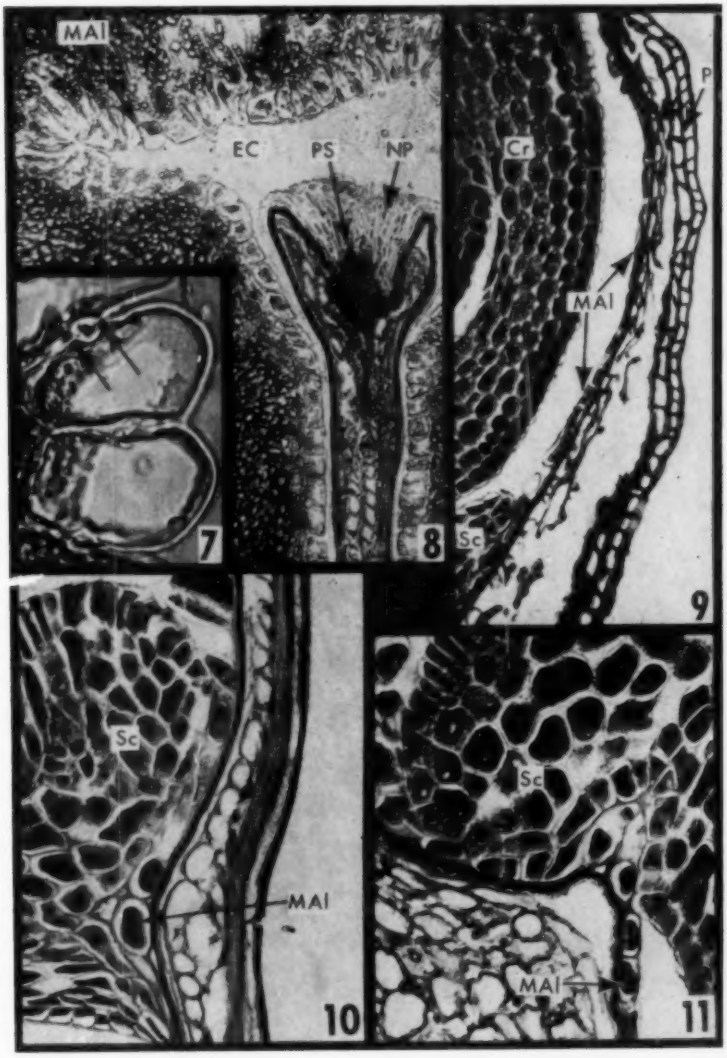


FIGS. 1-6. Fig. 1 — Transection through pericarp and aleurone layer (200×): P, pericarp; NE, nucellar epidermis; Al, aleurone layer; E, starchy endosperm. Fig. 2 — Surface view of aleurone layer (200×). Fig. 3 — Section through aleurone layer (500×): wall between adjacent cells at X; wall between aleurone cell and starchy endosperm cell at Y. Fig. 4 — Section through aleurone layer (500×); wall of center cell shown in face view. Figs. 5 and 6 — Sections of aleurone layer stained to show nuclei (200×): Note variation in thickness of aleurone cells in Fig. 5, and two superposed cells in Fig. 6.

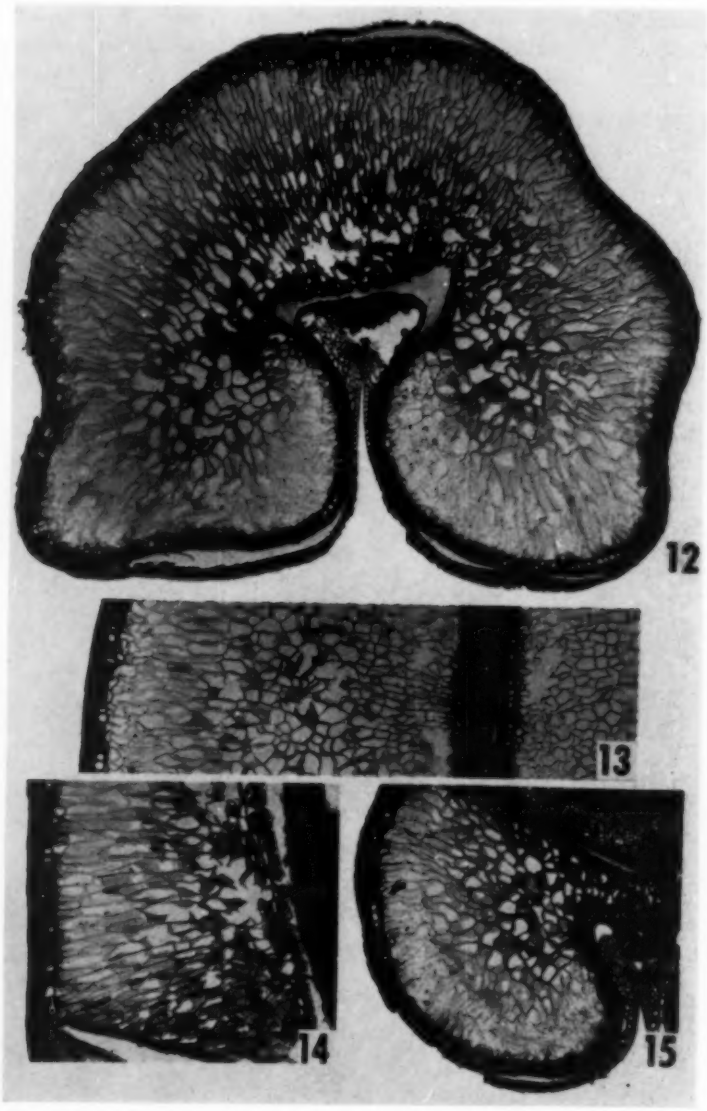
tions of the Pawnee wheat kernel the cells are often oblong or wedge-shaped, in contrast to the nearly square typical aleurone cells. Two superposed cells are frequently present. Each cell contains a nucleus surrounded by cytoplasm containing oil. The walls between adjacent cells contain conspicuous pits (Fig. 7). The walls between the modified aleurone cells and starchy endosperm cells are also pitted.

The modified aleurone layer, unaccompanied by starchy endosperm, extends over the edge of the scutellum and also over part of the embryonic axis (Fig. 9, MAl). Krauss (20) stated that the cells of the aleurone layer over the plumule are flattened and contain no aleurone

granules and that the aleurone layer is lacking over the projecting embryo tip. In sections of Pawnee wheat, scattered cells or groups of cells of the modified layer were found over the embryo as far down as



FIGS. 7–11. Fig. 7 — Two cells of modified aleurone layer in crease region (500×); arrows show pits in wall. Fig. 8 — Crease region in transection of kernel (100×): MAl, modified aleurone layer; EC, endosperm cavity; PS, pigment strand; NP, nucellar projection. Fig. 9 — Transection at level of epiblast (150×): P, pericarp; Cr, coleorhiza; MAl, modified aleurone layer; Sc, scutellum. Figs. 10 and 11 — Longisections through top and base of scutellum (200×): code as in Fig. 9.

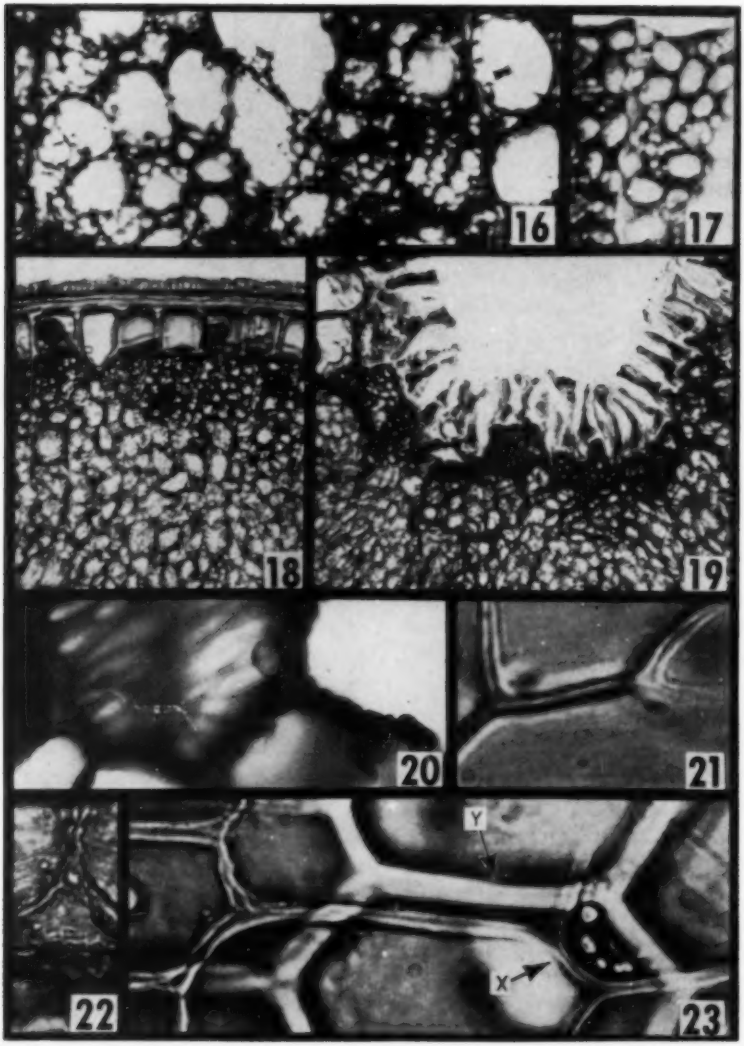


FIGS. 12–15. Sections of kernels in late soft dough stage of development, showing types and arrangement of endosperm cells (contents removed) (30×). Fig. 12 — Transection above germ. Fig. 13 — Longisection perpendicular to crease. Fig. 14 — Longisection parallel to crease. Fig. 15 — Transection through germ.

the level of the tip of the primary root. Thickness of the modified aleurone layer over the embryo was 8.8– 19 μ (21 measurements in 18 kernels). The mean, 13.4 μ, is less than one-third of the mean thickness of the typical aleurone cells measured. The modified aleurone layer over the surface of the embryo in a transection just above the tip of the epiblast is shown in Fig. 9. The aleurone cells form a practically continuous layer over the upper part of the embryo but both small and large intercellular spaces are present. The cells are thin-walled and each contains a nucleus and cytoplasm. The modified aleurone layer extends over the scutellum and into the embryonic cavity (Figs. 9–11).

Starchy Endosperm. The shape, size, and location of three types of starchy endosperm cells have been described and figured for a hard wheat, Manitoba variety (14). The same types of cells, peripheral, prismatic, and central, are present in Pawnee wheat. In size and in pattern of distribution they are similar to those described for the variety Manitoba. The peripheral cells form a row just inside the aleurone layer. They may be approximately isodiametric or elongated; elongation is usually toward the center of the endosperm. The prismatic cells, oriented with their long axes perpendicular to the surface of the kernel, lie roughly end to end and extend inward from the peripheral layer. The mass of prismatic cells reaches nearly to the crease in the back of the kernel (Figs. 12, 14) and part way to the cheek center in the sides and cheeks (Figs. 12, 13, 15). The central cells occupy the central portion of each cheek. Comparison of Figs. 12 and 13 shows that the central cells are similar in shape and size in trans- and longisections of the kernel. The peripheral cells of Manitoba wheat, measured in a transection of the kernel (14), were reported to be not over 60 μ long; the prismatic cells 128–200 μ by 40–64 μ; large central cells 120–144 μ by 80–120 μ; and small central cells 72–104 μ by 69–96 μ.

The starchy endosperm cells, with the exception of those in a compressed layer of endosperm that lies next to the scutellum, contain many starch granules that are embedded in a proteinaceous matrix rich in gluten-forming proteins. Starch granules were removed from prismatic cells (Fig. 16) and peripheral cells (Fig. 17) to make the proteinaceous network more evident. The spaces in the network indicate the sizes and arrangement of starch granules. Both prismatic and central cells contain large lenticular starch granules, oval to circular in outline, mostly 28–33 μ in diameter (30), with an upper limit of 50 μ (28 and others). The lenticular granules in the outermost prismatic cells are smaller than those in the inner cells. Packed between the large granules are numerous small, spherical to many-sided granules that vary from 2 to 8 μ (30) in diameter.



FIGS. 16–23. Figs. 16 and 17 — Proteinaceous network in prismatic cells and peripheral cells of starchy endosperm (500×). Figs. 18 and 19 — Transections showing starch granules in peripheral and interior endosperm cells (160×). Figs. 20–23 — Sections showing walls of starchy endosperm cells: Fig. 20, thick-walled cells near crease (500×). Figs. 21 and 22, thin-walled cells (1000×). Fig. 23, walls of subaleurone layer at X; walls of aleurone layer in background at Y (1000×).

Peripheral cells usually contain starch granules intermediate in size between the two groups already mentioned (3, 14, 19). These starch granules are rather uniform in size and often restricted to the outer

part of the cell (3, 19). A central area devoid of starch granules occurs in many peripheral cells of Pawnee wheat. A few small starch granules may be present in the peripheral cells in addition to those of medium size (Fig. 17).

The sharp contrast between the starch granules in peripheral cells and in cells lying closer to the interior of the kernel is easily observed (Figs. 18, 19); Fig. 18 shows starchy endosperm adjacent to the typical aleurone layer and Fig. 19 that adjacent to the modified aleurone layer. The peripheral cells, in these figures, appear darker than the others because they contain a higher percentage of proteinaceous material and have consequently taken up more of the Congo red dye used in staining the preparation. It has been known for many years that there is a gradient in protein content from the center of the starchy endosperm outward (8, 16, 23).

Developmental studies (1, 7, 15, 27) indicate that the nuclei of many starchy endosperm cells become irregular in shape as they are compressed between starch granules and finally degenerate and assume the form of a star-shaped structure or even a network.

The thickness of the walls of starchy endosperm cells varies in different parts of the kernel. In seven varieties of Pacific Northwest wheats most walls between starchy endosperm cells were less than 3 μ thick; but between cells near the aleurone layer they were about 4 μ thick, and near the crease, about 7 μ thick (21). Differences in thickness, and perhaps in composition, of the walls of starchy endosperm cells of Pawnee wheat are shown by the light- and dark-staining portions in the sections photographed for Figs. 12–15. A broad area of lighter-stained, seemingly thinner-walled cells lies between a narrow subaleurone region and a wide region that borders the endosperm cavity. Walls of some of the starchy endosperm cells are pitted; a photomicrograph of relatively thick-walled cells near the crease (Fig. 20) shows the beaded appearance of a wall in section at the right and the reticulated thickenings in a somewhat oblique surface view of a wall at the left. The thin-walled starchy endosperm cells are less evidently pitted (Figs. 21–23; note that magnification is twice that of Fig. 20). Comparison of walls of the subaleurone starchy endosperm layer with those between cells of the aleurone layer (Fig. 23) shows that the former are much thinner. There is a suggestion of the presence of pits in the thin walls shown in Fig. 22 and at the left side of Fig. 23. Guenther (15) reported that in the outer starchy endosperm the contents of adjacent cells are connected by delicate protoplasmic strands that pass through the walls.

The occurrence of floury and horny endosperm in wheat kernels of different varieties and within individual kernels has been discussed in

Part I. A higher relative protein content for vitreous kernels in comparison with mealy kernels has been reported (29). According to Alexandrov and Alexandrova (2) there is a characteristic difference between the small starch granules in cells of horny (vitreous) and of mealy (floury) endosperm. In vitreous endosperm they are rounded and separated by a considerable amount of proteinaceous material; in mealy endosperm they are closely packed and many-sided, thus leaving little space for nitrogenous material. The same authors reported that the walls of endosperm cells of hard wheats are thicker than those of soft wheats. Greer *et al.* (14) stated that they had not yet found evidence substantiating this report.

Significance of Structure

Aleurone Layer. The possible significance of the structure of the aleurone layer has been brought out in several publications. Cobb (8) suggested the use of structural characteristics of the aleurone layer in distinguishing varieties. He observed that usual date of maturity, nitrogen content, and other varietal characteristics were correlated with the percentage of area occupied by the cell contents of this layer when measured in surface view.

The aleurone layer is one of the tissues that must be traversed before water reaches the starchy endosperm. Water seems to pass through this layer easily. A recent article by Hinton (17) indicates that the aleurone layer is of little importance in regulating the rate at which water enters the starchy endosperm.

The variation in thickness of the aleurone layer has been considered to be one of the factors that make it difficult to detach starchy endosperm from bran during milling. Less frequent variation in thickness of adjacent aleurone cells in good-milling than in poor-milling varieties of Pacific Northwest wheats was reported by Larkin *et al.* (22). They found no evidence, however, that this difference was a major factor affecting milling quality. Variation in thickness of aleurone cells was noted in sections of Pawnee wheat, and it seems possible that this irregularity might tend to hold some starchy endosperm to the bran and thus to reduce the amount of flour extracted.

The present study has suggested a few hypotheses concerning the possible role of the modified aleurone layer during initial water uptake by immersed kernels. These will be discussed in Part IV (6).

Starchy Endosperm. Ways in which knowledge of the structure of the starchy endosperm cells and the size of the contained starch granules may be applied to practical problems are illustrated by the studies of several investigators.

Cobb (8) concluded that richness of the grain in nitrogenous matter is correlated with the presence of small cells filled with small starch granules. He suggested that the quality of flour derivable from the grain might be predicted from the size of the endosperm cells and of their starch granules as viewed in cross sections of the kernels.

The thickness of starchy endosperm cell walls may serve as a criterion of millability of the grain. Larkin *et al.* (21) reported a positive correlation in seven varieties of Pacific Northwest wheats between thin cell walls near the aleurone layer and a high milling score.

The texture of the endosperm seems to affect both the manner in which it is fractured during the grinding of the grain and the rate at which water is absorbed during tempering. A study of flours produced from hard and soft wheats (vitreous and mealy, by inference) led Berliner and Rüter (3) to conclude that the endosperm of hard wheats breaks between the endosperm cells while that of soft wheats tends to be pulverized so that few or no whole cells are left. Greer and Hinton (13) arrived at a similar conclusion from application of various fracturing techniques to hard and soft wheats.

Kent and Jones (19) described several possible ways in which fragments of endosperm could break to yield flour particles of correspondingly different types. Differences in the strength of the entire cell contents compared with that of the cell wall and of the strength of adhesion of cell wall to cell contents or to adjacent cell walls were suggested to explain different types of flour particles obtained in different mill streams.

Hinton (17) reported that in both hard and soft wheats, fully mealy endosperm was found to be twice as permeable to water as fully vitreous endosperm. He considered density of cell contents to be a factor affecting the rapidity of entrance of water. Cell wall thickness and composition may also be involved.

Application of knowledge of endosperm structure to an understanding of processing methods and problems may be expected to bring further advances in milling technology.

Acknowledgments

The authors are grateful to Kansas and Oklahoma Agricultural Experiment Stations for the samples of mature wheat and to Dr. Duncan Macmillan for the sample in the late soft dough stage.

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STRUCTURE OF THE MATURE WHEAT KERNEL
IV. Microscopic Structure of the Germ of Hard Red Winter Wheat[1]

DOROTHY BRADBURY, M. M. MACMASTERS, AND IRENE M. CULL[2]

ABSTRACT

The germ is a young plant composed of an embryonic axis partly enfolded by the shield-shaped scutellum. The plumule, composed of rudimentary stem and foliage leaves enclosed by the coleoptile, and the primary root covered by the coleorhiza make up the axis. Two pairs of secondary roots are present. One face of the scutellum is embedded in the starchy endosperm. On this surface the scutellar epidermis (the epithelium) is modified for the functions of food digestion and absorption. Through its action the foods stored in the endosperm are made available for resumption of growth of roots and shoot during germination of the seed. The epidermis of the scutellum adjacent to the plumule is heavily cutinized. The cuticle covering the embryonic axis is heavy only at the tip of the coleoptile and appears to be fragmentary or lacking over the end of the coleorhiza.

Cell characteristics that aid in the identification of embryo fragments in milled products are small size, thin walls, and contents of cytoplasm, nucleus, and oil droplets. Many of the cells, especially the scutellar parenchyma, contain small aleurone grains.

Consideration is given to the possible relation of the structure of the embryo and its adjacent tissues to the initial localized entrance of water into the kernel.

The germ, or embryo, of the wheat kernel, although highly nutritious, is usually excluded from flour because it contains oil that becomes rancid with age. The germ fraction of the milled products of wheat is ordinarily sold separately for human consumption or is incorporated in high-protein feeds. Some milling companies use patented processes to make white or light-colored flours that contain the germ. At present, extraction of oil from wheat germ is not carried on commercially to any great extent.

Knowledge of the structure of the germ and the tissues immediately surrounding it is important for several reasons. Improvement in methods for separating the germ will depend upon such knowledge. Famili-

[1] Manuscript received July 5, 1956. Presented at the 41st Annual Meeting, New York, May 1956.
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arity with the appearance of embryo tissues is useful for microscopic analyses in control laboratories of industries using wheat. It is, for example, valuable to feed microscopists because increasing emphasis is being placed on qualitative examination of feeds. An understanding of the detailed structure of the germ and neighboring tissues may throw some light upon the still unsolved question of the path and method of entrance of water into the starchy endosperm during tempering of the grain.

The gross morphology of the embryo has been discussed briefly in Part I (2). The main purpose of this paper is to consider the details of its structure. A glossary of botanical terms will be found on page 00.

Materials and Methods

Two samples of Pawnee variety hard red winter wheat, obtained from the Kansas and Oklahoma Agricultural Experiment Stations, respectively, provided the material for study. Frozen kernels and paraffin-embedded kernels were sectioned.

Frozen Material. Kernels were steeped for a few hours in water at room temperature or in an FAA fixative (2) at approximately 8° C. for 6–14 days. Sections were cut 16–50 μ thick. They were stained with 0.025% Congo red in an aqueous solution buffered to pH 8 with phosphate buffer (Figs. 1, 11 inset, 16, 18, 19, 21, 25), or with Sudan black B (Figs. 7, 15, 17), or with Sudan IV. Stained sections were mounted in the stain, in glycerol, or in the buffer solution.

Paraffin-Embedded Material. The most satisfactory slides were obtained from material fixed and dehydrated according to the first method described in Part II (3). Sections were cut 10–14 μ thick. The sections for Figs. 2–4, 6, 8–14, 20, 22, and 24 were stained by an iron alum-haematoxylin schedule and those for Figs. 5 and 23 were stained with safranin and fast green.

Paraffin-embedded material can be cut thinner than frozen material; thus the sections show details more clearly. In sections of paraffin-embedded material that have been dehydrated and mounted in balsam, the walls are thinner than in sections mounted in water or glycerol. In spite of efforts to avoid plasmolysis of the material, the contents of the cells shrank from the walls. This must be kept in mind when viewing Figs. 10, 12, 16, 20, 22, and 24, otherwise the space between the cell contents and the thin wall may be mistaken for part of the wall.

General Survey

The germ is a young, normally living, plant composed of a rudimentary root and shoot that together form the embryonic axis, and the

scutellum that functions as a storage, digestive, and absorbing organ. Parts of the embryo are labeled in Fig. 1. The stem apex, several embryonic foliage leaves, and an enclosing sheath (coleoptile) make up the shoot or plumule. Another sheath, the coleorhiza, encloses the primary

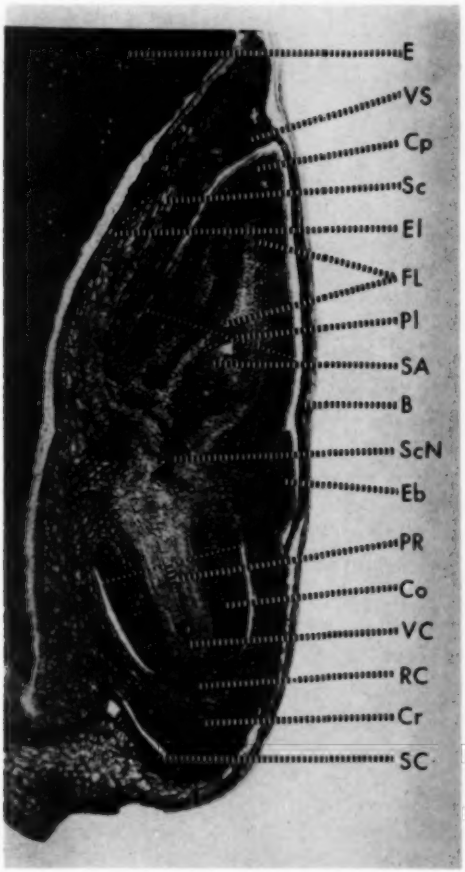


FIG. 1. Longisection of germ cut parallel to crease (44×). E, endosperm; VS, ventral scale; Cp, coleoptile; Sc, scutellum; El, epithelium; FL, foliage leaves; Pl, plumule; SA, stem apex; B, bran; ScN, scutellar node; EB, epiblast; PR, primary root; Co, cortex; VC, vascular cylinder; RC, root cap; Cr, coleorhiza; SC, seed coat.

root and two pairs of secondary lateral roots. The lateral roots do not show in a median longisection parallel to the crease. Below the plumule the scutellum and another lateral appendage, the epiblast, are attached to the embryonic axis. The scutellum is on the side toward the

endosperm and the epiblast on the opposite side. The epiblast is a small, scalelike structure composed of parenchyma cells. Because it probably has little morphological significance (1) it will not be discussed further.

Most cells of the germ are small and thin-walled, and contain a nucleus surrounded by cytoplasm. The cytoplasm of many of the cells contains oil and aleurone (protein) grains (22). The latter are especially abundant and conspicuous in the parenchyma cells of the scutellum. Only minute traces of starch are present in the dormant embryo (see 22).

A brief review of the nature and origin of some tissues of root and stem will aid in an understanding of the structure of the germ. Vascular tissues that conduct water and food through the plant are composed of xylem and phloem. In plants such as grasses and grains, the xylem and phloem are located in a central cylinder or ring in the root but they occur as scattered strands in the stem. In the leaves they form the veins.

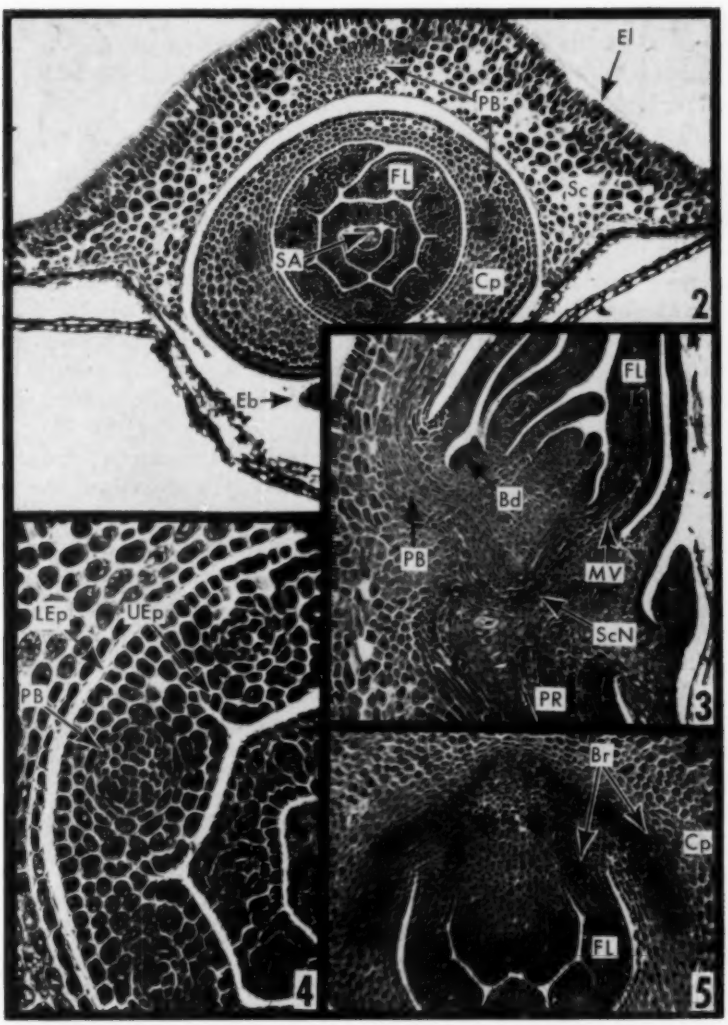
At the tip of the root and that of the stem (root apex and stem apex) there are certain cells that, while preserving their own identity, produce other cells destined to form numerous tissues characteristic of the mature plant. The new cells at first resemble the cells that produced them, and with them make up what is often called the apical meristem. Apical meristems are commonly composed of small, approximately isodiametric, thin-walled cells, each containing a nucleus and abundant cytoplasm. Under conditions suitable for growth the cells divide and their descendants undergo changes in size, shape, and nature of wall and contents that result in the formation of cells characteristic of different kinds of tissues. In the early stages of this differentiation, provascular tissue and protoderm, the forerunners of vascular tissues and epidermis respectively, can be distinguished. The provascular tissue usually differs markedly from the surrounding parenchyma in size, shape, and arrangement of cells and often in affinity for histological dyes.

The partially differentiated cells of root, stem, and leaf of the dormant wheat embryo were formed from apical meristems during the growth of the wheat kernel while it was still on the plant. With the return of conditions suitable for growth, the apical meristems of stem and root become active again and the seedling grows as a result of division, enlargement, and differentiation of cells.

Embryonic Axis

Plumule. The stem apex and several embryonic foliage leaves are visible in trans- and longisections through the plumule (Figs. 2, 3). In

the first two foliage leaves, the upper and the lower epidermis, and the provascular bundles are clearly differentiated from the remaining parenchyma cells (Fig. 4). Marked indentations are present on the up-



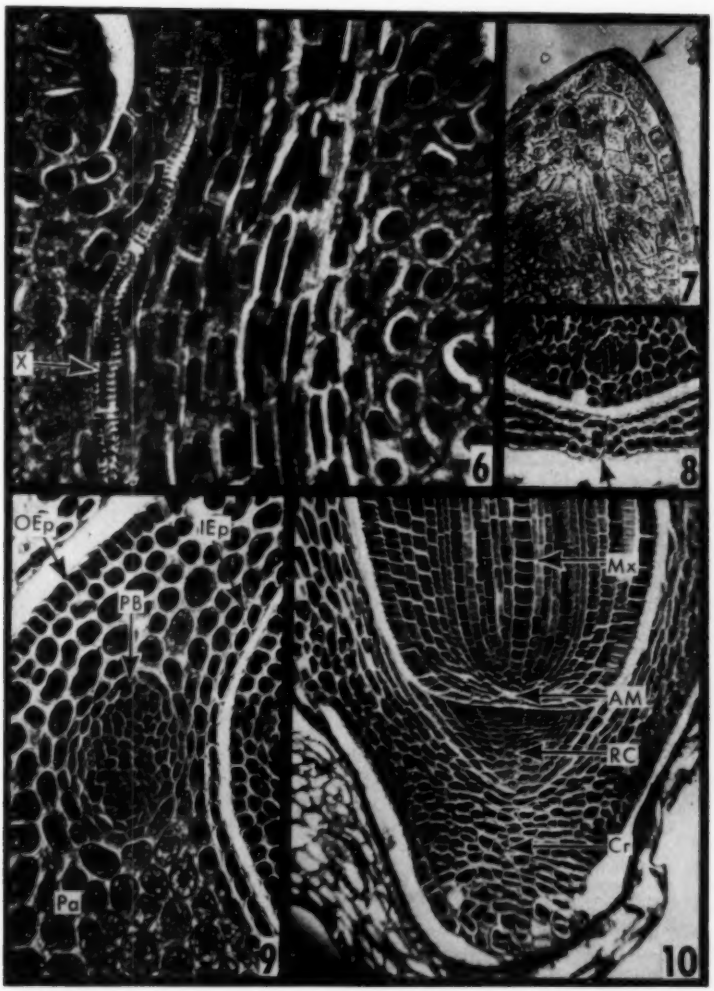
Figs. 2-5. Fig. 2 — Transection through plumule (60×): El, epithelium; PB, provascular bundle; Sc, scutellum; Cp, coleoptile; FL, first foliage leaf; SA, stem apex; Eb, epiblast. Fig. 3 — Longisection (60×); a branch of scutellar provascular bundle, PB, passes through scutellar node, ScN, and becomes midvein, MV, of first foliage leaf, FL; Bd, bud in axil of coleoptile; PR, primary root. Fig. 4 — Detail of foliage leaves (200×): UEp, upper epidermis; LEp, lower epidermis; PB, provascular bundle. Fig. 5 — Transection showing branches of scutellar bundle, Br, entering first leaf, FL, and coleoptile, Cp (60×).

per surface of the first leaf between the provascular bundles. Younger foliage leaves may be as yet only swellings on the stem apex although a third leaf is clearly defined in Fig. 2.

The first foliage leaf commonly has eleven veins and the second seven (22). The mid-vein (Fig. 3, MV) of the first foliage leaf is formed largely from a branch of the scutellar provascular bundle (Fig. 3, PB) that passes downward from the scutellum to the scutellar node (Fig. 3, ScN) and then upward through the vascular cylinder and into the leaf (1). Two or more of the lateral veins of the first foliage leaf are also formed by branches from branches of the scutellar provascular bundle (Fig. 5) (1). McCall (18) differs from Avery in his interpretation of detailed vascular anatomy of the wheat embryo and seedling. However, he, also, points out cross-axis provascular tissue at the first node (scutellar node or first node of Avery) from which a procambial strand on the anterior side enters the first foliage leaf and a procambial strand on the posterior side enters the scutellum. He also calls attention to the joining of two lateral bundles of the first foliage leaf with the coleoptile bundles and (at a lower level) of the coleoptile bundles with the procambial strand that enters the scutellum.

The provascular bundles are conspicuous in transections because of the small diameter of their cells (Fig. 4) and in longisections because their cells are slender and elongated (Figs. 3, 6). For the most part, cells of the provascular bundles of the embryo are immature; that is, they have not attained size, shape, wall-thickness, or other features characteristic of mature xylem and phloem. However, the distinctive spiral wall-thickenings of xylem elements can be seen in Fig. 6 in a bundle at the base of the first foliage leaf.

The coleoptile is a roughly cone-shaped sheath (Fig. 1) about 1–1.25 mm. long (22) that encloses the embryonic foliage leaves. It is covered by a cuticle that is very delicate except over the tip (Fig. 7). Near its tip on the side away from the scutellum there is a small pore (Fig. 8) through which the foliage leaves emerge during germination of the seed. The coleoptile, seen in transection, is somewhat elliptical in shape, with the long axis parallel to the scutellum (Fig. 2). It is from 6 to 12 cell layers thick; the thickest parts are at the poles of the ellipse. An outer and inner epidermis, each composed of a single cell layer, are evident (Fig. 9). Thin-walled parenchyma cells make up the inner tissue except for the two provascular bundles (Figs. 2, 9). These bundles, one in each thickened part of the coleoptile, run throughout its length and near its tip turn toward each other and meet in a V-shaped, backward-turning curve (22). They arise as branches from branches of the scutellar bundle (Fig. 5). Although two provascular bundles are



FIGS. 6-10. Fig. 6 — Longisection of provascular bundle at base of first foliage leaf, showing xylem elements with spiral wall-thickenings, X (500×). Fig. 7 — Longisection through tip of coleoptile; ribbon of cuticle shown lying flat (arrow) (160×). Fig. 8 — Transection through plumule; pore of coleoptile at arrow (150×). Fig. 9 — Portion of coleoptile (detail of Fig. 2 — 200×); OEp, outer epidermis; IEp, inner epidermis; PB, provascular bundle; Pa, parenchyma. Fig. 10 — Longisection through tip of primary root (100×); Mx, metaxylem; AM, apical meristem; RC, root cap; Cr, coleorhiza.

usually present in the coleoptile of the bread wheats, the frequent occurrence of more than two in some wheat species has been reported (14, 19, 22).

There has been much dispute over the coleoptile. Many consider it

to be the first leaf of the plumule; a recent publication by Reeder (24) supports this view. A bud is usually present in the axil of the coleoptile (Fig. 3); this also indicates that the coleoptile may be a modified leaf (1). Stomata in an immature stage of development are present in the epidermis of the coleoptile of the resting embryo.

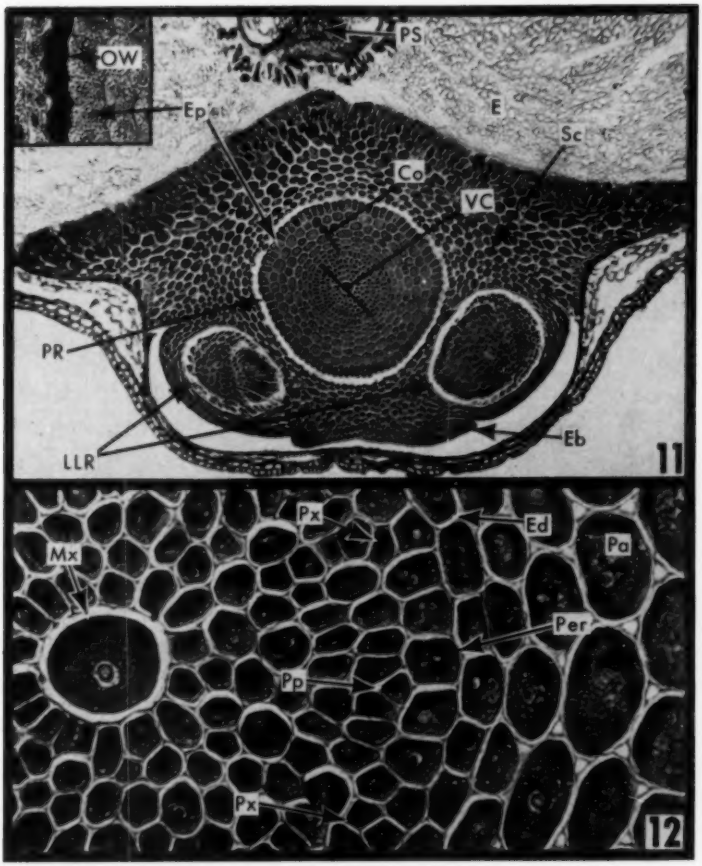
Internodes. Internodes are regions of the stem between successive nodes (places where leaves originate). They are extremely short in the wheat embryo. The internode between the attachments of coleoptile and first foliage leaf is shown in a slightly oblique section in Fig. 13. In this internode, which elongates greatly during germination, there is a scattered arrangement of provascular bundles characteristic of a transition from root structure to stem structure. Details of the vascular system of the nodes and internodes are more easily studied in the seedling plant. They have been fully described by Avery (1), McCall (18), and Percival (22).

Primary Root. The primary root arises below the scutellar node and points downward toward the base of the kernel (Fig. 1). In its apical meristem (Fig. 10, AM) there are three tiers of cells that give rise to root tissues (22). One gives rise to the thimble-shaped root cap; another to the cells that form the epidermis and the cortex of the root; and the third to the central vascular cylinder in which conducting elements, xylem and phloem, differentiate.

The root cap (Fig. 10) is easily recognized by its location at the tip of the root and by the arrangement of the rows of cells composing it.

The epidermis, and the boundaries of the cortex and the central vascular cylinder of the root, can be distinguished in trans- and longisections, even though the cells of the tissues have not reached maturity (Fig. 11). Four to six layers of thin-walled parenchyma cells with very small intercellular spaces lie between the epidermis and the innermost layer of the cortex, the endodermis. The outer walls of the young epidermal cells are much thickened (inset, Fig. 11) and are reported to be mucilaginous (22).

The tissues of the central vascular cylinder are shown in Fig. 12. The outermost layer, the pericycle, lies just within the endodermis of the cortex. Protoxylem cells are spaced at rather regular intervals just beneath the pericycle. The number of groups of protoxylem cells is most commonly seven or eight (1). Protophloem cells alternate with the protoxylem. The large cell in the center of the vascular cylinder is a metaxylem element, called *metaxylem* because it is later than protoxylem in maturing. That this cell is one of a longitudinal row of similar cells can be seen in Fig. 10. The primary root of the embryo usually has a single central row of large metaxylem elements but occasionally

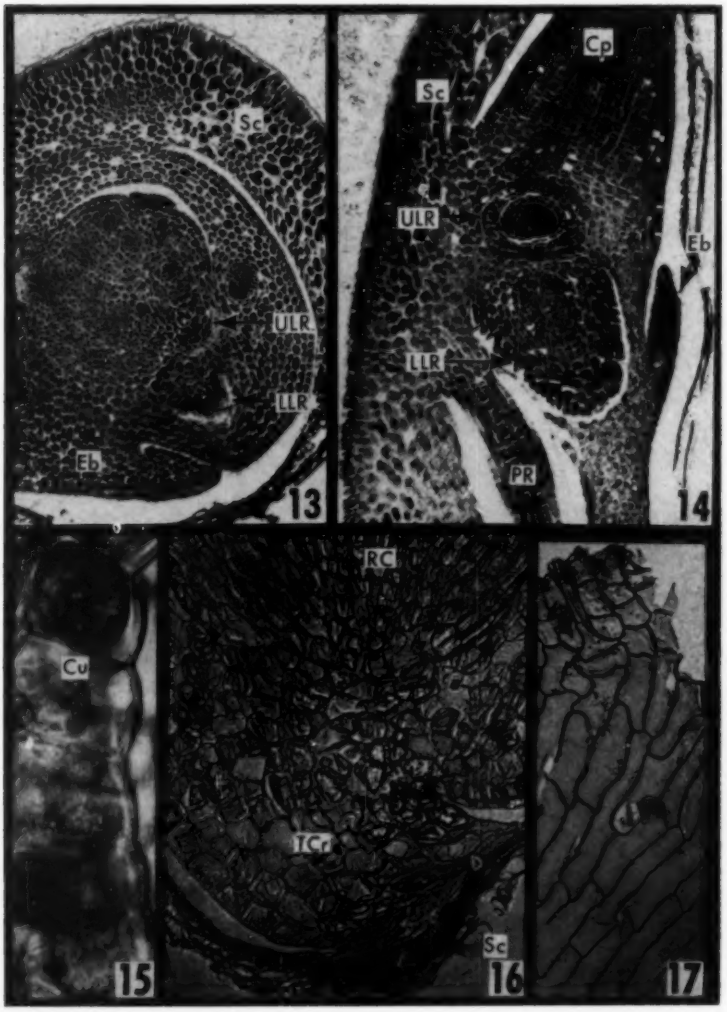


FIGS. 11, 12. Fig. 11 — Transection through embryo at level of roots (60×): PS, pigment strand; E, endosperm; Sc, scutellum; PR, primary root; LLR, lower lateral roots; Ep, epidermis; Co, cortex; VC, vascular cylinder; Eb, epiblast. In inset (200×) the thick outer walls, OW, of epidermal cells have been deeply stained. Fig. 12 — Portion of transection of primary root (500×): Pa, parenchyma of cortex; Ed, endodermis; Per, pericycle; Px, protoxylem; Pp, protophloem; Mx, metaxylem.

another row, similar but smaller in diameter, is present. At this stage of development, the rest of the vascular cylinder is composed of parenchyma cells.

Two pairs of secondary lateral roots are present in the embryo. The lower pair arises from the embryonic axis at about the level of the base of the epiblast. One of the upper and one of the lower pair originate on each side of the axis and slant downward toward the face of the embryo. They are not as large as the primary root; the upper pair is especially small. The positions of these lateral roots relative to each

other and to the primary root may be seen in trans- and longisections of the embryo (Figs. 11, 13, 14). In general structure, the lateral roots resemble the primary root.



FIGS. 13-17. Fig. 13 — Slightly oblique transection through internode above the attachment of the coleoptile (60×): Sc, scutellum; ULR, upper lateral root; LLR, lower lateral root; Eb, epiblast. Fig. 14 — Longisection of embryo through one of each pair of lateral roots (60×): Cp, coleoptile; PR, primary root. Rest of code as in Fig. 13. Fig. 15 — Longisection through epidermis on upper part of coleorhiza (1000×): Cu, cuticle. Fig. 16 — Longisection through tip of coleorhiza, TCr (200×): RC, root cap; SC, seed coat in micropylar region. Fig. 17 — Portion of cuticle from epidermis on concave surface of scutellum (200×).

Coleorhiza. The coleorhiza sheathes the primary root. On the side next to the endosperm it merges with the scutellum (Fig. 1). The coleorhiza is composed of parenchyma cells bounded by inner and outer epidermal layers and lacks provascular tissue. The outer epidermis is covered with a cuticle (Fig. 15) to within a short distance of the tip. In the present study the cuticle was never followed with certainty over the tip, although in some preparations fragments of cuticle appeared to be present there.

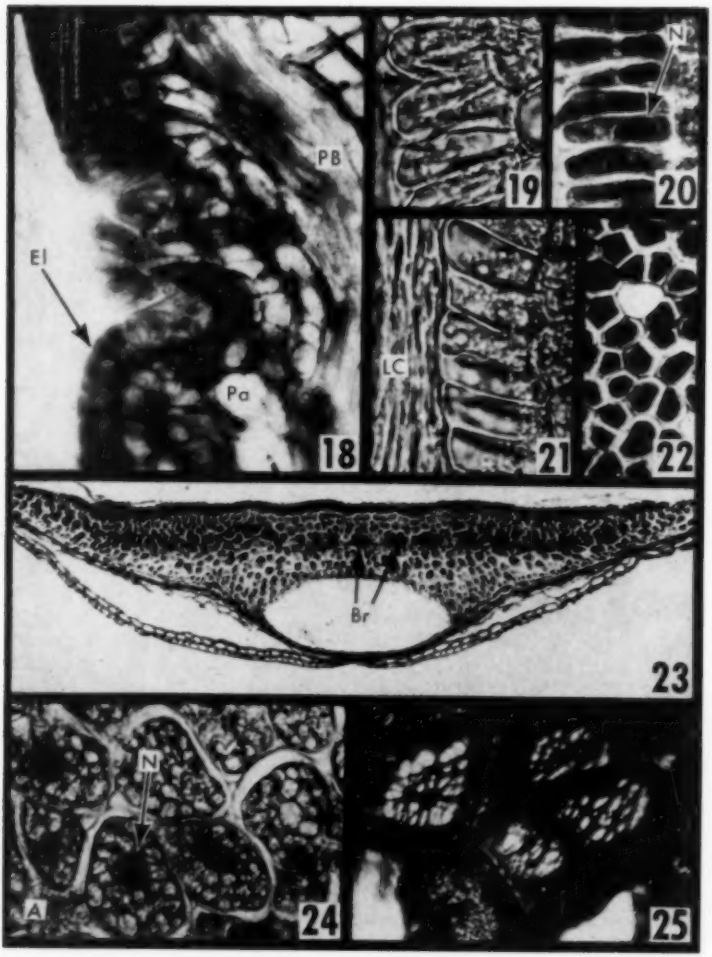
The tip of the coleorhiza has a smooth, even contour on the side nearest the center of the kernel but is characteristically and conspicuously indented on the opposite side (Fig. 16). The cells are slightly thicker-walled in the tip region than elsewhere in the coleorhiza. A layer of compressed, usually empty cells, instead of a well-defined epidermis, covers the tip and lies next to the seed coat in the micropylar region (Fig. 16) (see Part II). It is possible that the layer of compressed cells is comparable to the "embryonic appendage" of oats described by Collins (6).

Scutellum

The scutellum stores food for the embryonic plant and at the time of germination becomes a digesting and absorbing organ (22) that transfers food from the adjacent endosperm to the growing parts of the embryo. The scutellum is shield-shaped; its convex surface lies next to the endosperm and its opposite, concave surface partly enfolds the embryonic axis (Figs. 1, 2). The slight projection near the tip of the scutellum is called the ventral scale (Fig. 1).

Epidermis. Most of the cells of the epidermis in the area adjacent to the coleoptile are elongated with the long axis of the scutellum. They are covered by a rather thick cuticle. A portion of isolated cuticle is shown in Fig. 17. The pattern of the epidermal cells is strongly marked on the cuticle—probably by the presence of "pegs" that were embedded in the radial walls of the epidermis as in corn (29). The pattern of short cells visible in Fig. 17 is on the portion of the cuticle that was close to the rim of the concave surface of the scutellum.

The epidermis of the scutellum adjacent to the endosperm is modified to form a layer of secreting cells, the epithelium (Figs. 1, 2). In bread wheats the surface of the scutellum is essentially free of the invaginations, or "glands," so numerous and conspicuous in the scutellum of the corn embryo (see 29). Only a few slight invaginations are sometimes present near the tip of the scutellum (Fig. 18). The presence of glandlike cavities in some other wheat species has been reported (22).



FIGS. 18–25. Fig. 18 — Longisection through upper part of scutellum (200×): PB, provascular bundle; El, epithelium; Pa, parenchyma. Figs. 19, 20, 21 — Longisections of epithelium (500×): Arrow (Fig. 19) points to space between ends of adjacent cells. N, nucleus; LC, layer of crushed endosperm cells. Fig. 22 — Cross section of epithelium (500×): Contents of cells contracted or lost. Fig. 23 — Transection through embryo near tip of plumule (60×): Plumule tissue has dropped out of section. Br, small branches of provascular bundle of scutellum. Fig. 24 — Parenchyma cells of scutellum (500×): N, nucleus in mass of cytoplasm; A, aleurone grain. Fig. 25 — Surface view of walls of parenchyma cells of scutellum showing thin porelike areas (500×).

The individual cells of the epithelium are cylindrical, 35 — 40 μ long by 8–10 μ wide (22), and their length runs perpendicular to the surface of the scutellum. Occasionally one is divided into two by a transverse wall. The cells are not arranged in any regular order (Fig. 22). Some

taper toward the tip and may be separated from their neighbors through a considerable portion of their length (Fig. 19, at arrow); others are closely appressed throughout their length (Fig. 21). Each cell contains a nucleus (Fig. 20). Oil is present as fine droplets in the cytoplasm. The cells of the epithelium are in intimate contact with a layer of crushed endosperm cells (Fig. 21, LC).

Provascular Bundle. The provascular bundle of the scutellum is composed of numerous elongated protoxylem and protophloem cells which are smaller in diameter than the surrounding parenchyma cells. The bundle, consequently, is conspicuous in both trans- and longisections (Figs. 2, 3). It extends from the scutellar node into the upper part of the scutellum. In some embryos, xylem elements similar to those shown in Fig. 6 were observed throughout most of the length of the provascular bundle. Near the tip of the scutellum the bundle divides into many small branches that curve outward laterally and also toward the convex surface of the scutellum and then extend downward for a considerable distance (22). These numerous small branches are shown in a transection of the embryo near the tip of the plumule (Fig. 23; the plumule has dropped out of the section).

Parenchyma. The polyhedral parenchyma cells that compose the body of the scutellum vary considerably in size (Fig. 2). Only small intercellular spaces are present. The cells contain, in addition to nucleus and cytoplasm, stored food in the form of oil droplets and small masses of protein known as aleurone grains (Fig. 24). A surface view of the walls of these cells shows reticulate (netlike) wall thickenings and thin porelike areas (Fig. 25; the section photographed had been treated with alkali to remove the cell contents, and wall thickness may have been affected by the treatment).

Discussion

Industrial Microscopy. The accompanying photomicrographs should provide useful reference material on embryo structure for industrial microscopists. Sections of the embryo mounted in glycerol or phosphate buffer solution (Figs. 1, 7, 15, 16, 18, 19, 21) resemble fragments present in milled products more closely than do the sections of paraffin-embedded material that were dehydrated and mounted in balsam. The latter, however, show details more clearly. In general, embryo tissues are characterized by small, thin-walled cells filled with living substance that contains oil. Some of the cells are packed with small aleurone granules. Absence of appreciable amounts of starch from wheat scutel-

lum serve to distinguish wheat germ from corn germ, in which the scutellum contains small starch granules.

Absorption of Water and Degermination. Details of structure of the embryo and surrounding tissues have a bearing upon the practical problems of degermination of the grain and of entrance of water in tempering or conditioning treatments. Because the embryo is a complete, distinct organ of the seed, there is a natural line of cleavage between it and the adjacent endosperm on one side and the overlying bran on the other. However, the similarity in appearance between the interface of scutellum and endosperm in wheat and in corn suggests that there may be in wheat a "cementing layer" between epithelium and compressed endosperm cells similar to that reported for corn (29). Water used in tempering probably helps to weaken the bond between germ and endosperm as it does in corn. The living contents of the cells of the embryo are highly hydrophylic, and the embryo has a greater water-absorbing capacity than a piece of endosperm of similar size (7). This differential absorption may facilitate degermination. It should be borne in mind that heat of a degree and duration sufficient to kill the cells would result in changes in water absorption, because death alters the physical nature of the cell contents and the semipermeability of its limiting membranes.

Entrance of water into the kernels of cereal grains was studied first by those interested in the physiology of seed germination (6, 20, 26, 30); later by those concerned with the tempering and conditioning of grain for milling (5, 8, 9, 10, 11, 12, 13, 15, 21, 25, 27, 28). On the basis of these investigations the following statements concerning the entrance of water into immersed wheat kernels at temperatures not over 50° C. can be made with considerable assurance.

1. The pericarp takes up water very quickly.

2. Entrance of water into the interior of the kernel is delayed by slow passage through the semipermeable covering formed about the seed by the seed coat and pigment strand.

3. First evidence of the entry of water into the starchy endosperm occurs in the vicinity of the embryo after about an hour's immersion of the kernel.

In considering the entrance of water into the wheat kernel it is necessary to distinguish between entrance into the pericarp and entrance into the starchy endosperm. Methods using iodine for the detection of moisture are based upon color reactions with starch, and starch is not present in the pericarp. It seems likely that the capillary system referred to by Fritsch (9, 10) is formed of xylem elements and inter-

cellular spaces leading from the attachment region into the pericarp. If this interpretation is correct the system allows for permeation of the pericarp only.

Several investigators (5, 6, 11, 28) reported that, upon immersion of wheat (or barley) kernels in water, moisture first became apparent in the starchy endosperm in a circular or ringlike region around the edge of the scutellum as the kernel is viewed from the germ surface. One may assume that uninjured kernels were used in their experiments. Hinton (13) considered these experiments to be of little practical significance since Ugrimoff (28) reported visible entry of water only after 1 hour. Wheat, during washing, is normally in contact with excess water for only a few minutes. However, the most commonly used methods for determining the entrance of water are not delicate enough to detect the first small changes in moisture content. Water from a solution of potassium thiocyanate used to determine entrance of moisture probably permeates the tissues of the kernel before the thiocyanate does. With the iodine vapor method, moisture content only a little below 15–16% can be detected in the starchy endosperm by shades of yellow, orange, and brown (25, 28). Jones and Campbell (16) have only recently developed a method for determination of the moisture content of small endosperm particles from vitreous kernels to within $\pm 0.3\%$ over a range of 9–20%. It is possible that some localized or general entrance of small amounts of water into the endosperm may occur rapidly under the conditions of commercial tempering. Only the application of a method that can detect small changes in moisture content can clarify the question.

Structure of the germ and its surrounding tissues suggests several seemingly feasible paths for the initial entrance of water into the starchy endosperm of immersed kernels:

1. Surface of the seed coat over the embryo. The outer cuticle of the seed coat has been reported to be thinnest over the embryo (23); it is possible that water passes through this portion of the seed coat more quickly than elsewhere. Water entering here would naturally move into the nucellar layer and the modified aleurone layer. For discussion of passage into the starchy endosperm see 3, below.

2. Micropylar region of seed coat through coleorhiza and root to the scutellum. At the micropylar region in red wheat the outer and inner cuticles of the seed coat are extremely thin or, perhaps, completely lacking (3, 17, 23). This area appears to offer a comparatively easy path for entrance of water. Presumably water could then pass into the embryo through the tip of the coleorhiza and into the root. The

coleorhiza and root are joined to the scutellum and the convex surface of the scutellum borders on the layer of compressed cells of the starchy endosperm.

3. Micropylar region of seed coat into embryonic cavity and modified aleurone layer. Although contact between the seed coat at the micropylar region and the compressed cells of the tip of the coleorhiza is very close in the dry kernel, it seems likely that water entering through the seed coat at this region can pass into the embryonic cavity. In fact there is some evidence for this assumption. It was found that in a high percentage of kernels with unbroken pericarp, immersion for 6 hours in a 0.05% aqueous solution of Congo red resulted in the coloring of the modified aleurone cells lining the upper part of the embryonic cavity. Assuming an embryonic cavity filled with water, the thin-walled modified aleurone cells appear to offer a passageway around the edge of the scutellum to the starchy endosperm; see Figs. 9, 10, and 11 in Part III.

4. Base of pigment strand through the nucellar projection to the modified aleurone layer. This pathway was considered by Krauss (17). She concluded that the lignified layer of the cell walls of the corky pigment strand offered a pathway for penetration of water. The base of the pigment strand is in contact externally with the spongy tissue of the attachment region; toward the inside, it contacts the nucellar projection that joins either the modified aleurone layer or, in a small area, the scutellar epithelium. The cells of the modified aleurone layer bordering the lower part of the scutellum are thin-walled (Part III); they may offer little resistance to the passage of water.

Consideration of these hypothetical routes for the passage of water from pericarp through pigment strand or seed coat to the starchy endosperm may be of help to those who are studying the entrance of water into the interior of the wheat kernel. As pointed out by Ugrimoff (28), conditions that prevail when individual kernels are soaked in water for experimental work are quite different from those that exist in the large-scale moistening of grain for processing. Worthy of note, also, are the reports of Schäfer (25) and Buré (5) that the initial localized entrance of moisture in the vicinity of the germ is not nearly as apparent when the kernels are immersed in water at 70°–100° C. or are treated with steam as when they are moistened at a lower temperature.

Acknowledgments

The authors are indebted to Kansas and Oklahoma Experiment Stations for the wheat samples used and to J. E. Hubbard for sampling.

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GLOSSARY

Apical. Refers to the apex or tip of a structure.
Apical meristem. A region located at tip of root or stem and concerned with the more or less continuous formation of new cells and tissues.
Attachment region. As applied to wheat kernel: the basal area along which the kernel was formerly attached to the parent plant.
Bran. As applied to wheat kernel: the outermost tissues, including pericarp, seed coat, nucellar layer, and aleurone layer.
Cheeks. As applied to wheat kernel: the two parts, one on each side of the crease, which project toward the front of the kernel.
Coleorhiza. The structure which sheathes the primary root of the embryo of grasses.
Cortex. A cylindrical layer, composed generally of parenchyma tissue, surrounding the central core which contains the vascular tissues.
Cuticle. A layer of cutin which is firm, continuous, and relatively water-impervious.
Cuticularized. Covered with a cuticle.
Cytoplasm. The portion of the living substance or protoplasm of a cell exclusive of the nucleus and some other specialized structures.
Dorsal. As applied to wheat kernel: the back, where the germ is located.
Endodermis. A layer of cells separating the central vascular cylinder from the cortex. It is frequently considered to be the innermost layer of the cortex.
Endosperm cavity. The cavity which is present adjacent to wheat endosperm in the vicinity of the crease.
Epidermis. The surface layer of a plant organ.
Epithelium. A tissue composed of secretory cells.
Hypha (pl., *hyphae*). A single filament of the body of a mold.
Hypodermis. One or several layers of cells, frequently thick-walled, located just beneath the epidermis and serving to reinforce the latter.
Integument. An enveloping cloak or covering.
Isodiametric. As applied to cells: diameter approximately equal in all directions.
Meristem (adj., *meristematic*). A tissue which more or less continuously produces new cells and tissues.
Micropyle. An opening in the integuments through which the pollen tube penetrates the ovule (forerunner of the seed). The micropyle may or may not be present in the mature seed.
Nucellar epidermis. The outermost cell layer of the nucellus, the central part of the ovule (forerunner of the seed). The nucellar epidermis persists in the mature wheat kernel.
Nucellar projection. In the wheat kernel, a strand of tissue which lies interior to the pigment strand and runs parallel to it.
Nucleus (pl., nuclei). A highly organized, usually spherical or disk-shaped part of the living contents of a plant cell. It plays an important part in regulating the activities of the cell and it contains the hereditary units.
Parenchyma. Unspecialized vegetative tissue.
Pericarp. The fruit coat which surrounds and encloses the seed.
Pericycle. A tissue which forms the outermost layer of the central vascular cylinder.
Phloem. A conductive tissue associated with the xylem and serving mainly in the transport of foods.

Pigment strand. A strand of cells which extends nearly the length of the wheat kernel interior to the pericarp tissue in the bottom of the V-shaped crease. In red wheats the cells are deeply pigmented.

Pit. Used in a nontechnical sense, a thin area or depression in a cell wall. Pits occur generally in pairs on the opposite sides of the wall separating adjacent cells. The thin area in the wall forms the floor of both pits.

Plumule. The rudimentary shoot of a plant embryo. In grasses, the plumule is composed of stem apex and embryonic leaves surrounded by a sheath, the coleoptile.

Protoderm. A young, meristematic tissue which becomes the epidermis of a plant organ.

Protophloem. The first phloem cells to mature from an apical meristem. In the germ, these cells are still immature.

Protoxylem. The first xylem cells to mature from an apical meristem. In the germ, these cells are usually still immature.

Provascular bundles. Vascular strands in which the cells of xylem and phloem are only partially differentiated.

Provascular tissue. A young, meristematic tissue which gives rise to the vascular tissues, xylem and phloem.

Scutellar node. The region of the embryonic axis at the level of attachment of the scutellum (seed leaf or cotyledon.)

Stoma (pl., *stomata*). An opening in the epidermis through which gaseous exchange occurs between the atmosphere and intercellular spaces. The term is also applied to the opening together with specialized cells surrounding it.

Suberization. A change to a corky composition resulting from impregnation with suberin.

Vascular bundles. Strands composed of the conducting tissues (xylem and phloem).

Ventral. As applied to wheat kernel: the front in which the crease occurs.

Xylem. A conductive tissue associated with the phloem and serving in the transport of water and dissolved substances.

COMMUNICATION TO THE EDITOR

Revised data for Cereal Chemistry 32: 463–471 (1955).

DEAR SIR:

Attention of the authors has been drawn to certain errors in the paper, "Chopin alveograph studies. I. Dough resistance at constant sample deformation," by I. Hlynka and F. W. Barth, cited above. These include a stenographic inversion of the last two figures for the value of the volume of the dough membrane given on p. 466, and a displacement of the eighth entry in the second column of Table II from the fifth position in rank order.

TABLE I

CALCULATED DATA ON ALVEOGRAPH BUBBLES OF VARYING SIZE

HEIGHT OF BUBBLE	RADIUS OF SPHERE	AREA OF BUBBLE SECTION	VOLUME OF BUBBLE SECTION	THICKNESS OF BUBBLE WALL
cm.	*cm.*	$cm.^2$	$cm.^3$	*mm.*
0.60	6.65	25.1	7.3	2.38
0.70	5.79	25.5	8.6	2.34
0.80	5.16	25.9	9.8	2.31
1.00	4.31	27.1	12.5	2.21
1.20	3.78	28.5	15.3	2.10
1.50	3.29	31.0	19.7	1.93
2.00	2.91	36.6	28.1	1.63
2.50	2.77	43.5	38.1	1.37
3.04	2.77	52.9	50.9	1.13
3.82	2.91	69.8	75.1	0.86
5.08	3.29	105.0	129.5	0.57
6.36	3.78	151.1	210.9	0.40
7.62	4.31	206.3	322.9	0.29
9.52	5.16	308.7	565.7	0.19
10.88	5.79	395.8	804.5	0.15
12.70	6.65	530.6	1224.7	0.11

Advantage is being taken of this opportunity to increase the precision of the initial values in the fourth column of Table II by a more accurate graphical evaluation, and to recheck all the data on the basis of 5.98 cm^3 for the volume of the dough membrane.

The authors regret their oversight and present revised Tables I and II.

TABLE II

TIME-DEPENDENCE OF ALVEOGRAM AND DOUGH BUBBLE DIMENSIONS

TIME	DISTANCE ON ALVEOGRAM	VOLUME OF BUBBLE	AREA OF BUBBLE	THICKNESS OF BUBBLE WALL
sec.	cm.	$cm.^3$	$cm.^2$	mm.
0.2	0.12	5	24	2.14
0.5	0.29	12	27	1.87
1.0	0.59	25	34	1.42
2.0	1.18	50	52	1.05
3.0	1.77	75	70	0.82
4.0	2.36	100	86	0.69
5.0	2.95	125	103	0.58
7.0	4.12	175	132	0.45
9.0	5.30	225	157	0.38
10.0	5.89	250	170	0.35
12.0	7.07	300	195	0.31
14.0	8.25	350	218	0.27
15.0	8.84	375	228	0.26

October 15, 1956

I. HLYNKA
Grain Research Laboratory,
Board of Grain Commissioners
for Canada,
Winnipeg 2, Manitoba

Cereal Chemistry

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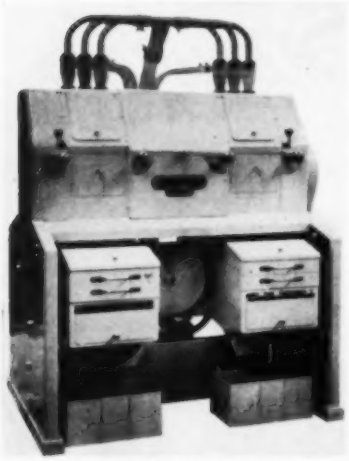

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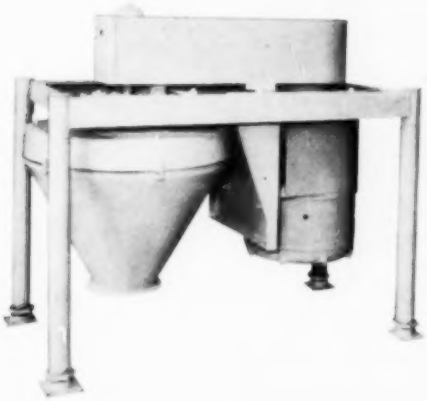

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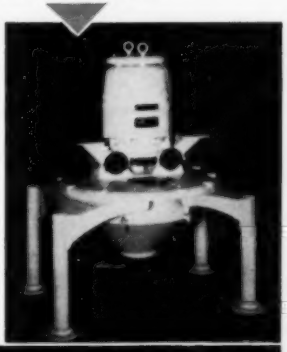

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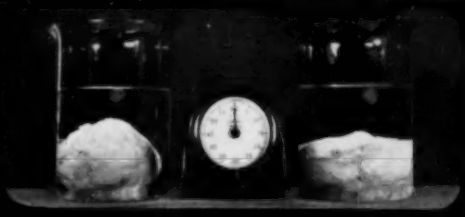

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